RELATIONSHIP BETWEEN SERUM ZINC LEVEL AND MICROVASCULAR COMPLICATIONS IN PATIENTS WITH TYPE 2 DIABETES

A Dissertation Submitted to

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For the Award of the Degree of

M.D. (GENERAL MEDICINE) - BRANCH – I

GOVERNMENT KILPAUK MEDICAL COLLEGE

CHENNAI

MAY 2019

BONAFIDE CERTIFICATE

This is to certify that "**Relationship Between Serum Zinc Level and Microvascular Complications in Patients with Type 2 Diabetes"** is a bonafide work done by **Dr. P.KALPANA** Post graduate student, Department of General Medicine, Kilpauk Medical College, Chennai-10, under my guidance and supervision in partial fulfillment of rules and regulations of the Tamil Nadu Dr. M.G.R Medical University, for the award of M.D. Degree Branch I (General Medicine) during the academic period from MAY 2015 To MAY 2019.

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DECLARATION

I solemnly declare that this dissertation **"RELATIONSHIP BETWEEN SERUM ZINC LEVEL AND MICROVASCULAR COMPLICATIONS IN PATIENTS WITH TYPE 2 DIABETES"** was prepared by me at Government Kilpauk Medical College and Hospital, Chennai, under the guidance and supervision of **Prof. Dr. K.E. GOVINDARAJULU, M.D,** Professor of General Medicine, Department of Internal Medicine, Government Kilpauk Medical College and Hospital, Chennai. This dissertation is submitted to **The Tamil Nadu Dr. M.G.R. Medical University, Chennai** in partial fulfillment of the University regulations for the award of the degree of **M.D. Branch I (General Medicine)**.

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The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "RELATIONSHIP BETWEEN SERUM ZINC LEVEL AND MICROVASCULAR COMPLICATIONS IN PATIENTS WITH TYPE 2 DIABETES " submitted by Dr.P.Kalpana, Post Graduate in General Medicine, Govt. Kilpauk Medical College, Chennai-10.

The Proposal is **APPROVED.**

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

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ABBREVIATION

DM	-	DIABETES MELLITUS
Zn	-	ZINC
MT	-	METALLOTHIONEIN
ROS	-	REACTIVE OXYGEN SPECIES
IGT	-	IMPAIRED GLUCOSE TOLERANCE
		IFG IMPAIRED FASTING GLUCOSE
IRS	-	INSULIN RECEPTOR SUBSTRATES
TBARS	-	PLASMA THIOBARBITURIC ACID REACTIVE SUBSTANCES
SOD	-	SUPEROXIDE DISMUTASE
MDA	-	MALONDIALDEHYDE
Nrf2	-	NUCLEAR FACTOR
GLUT 4	-	GLUCOSE TRANSPORTER
AGE	-	ADVANCED GLYCATION END PRODUCTS
DR		DIABETIC RETINOPATHY
DN	-	DIABETIC NEPHROPATHY
DPN	-	DIABETIC PERIPHERAL NEUROPATHY
AMPK	-	ACTIVATED PROTEIN KINASE
GPx	-	GLUTATHIONE PEROXIDASE
ZNT8	-	ZINC TRANSPORTER
CVD	-	CARDIOVASCULAR DISEASE
HiAPP	-	HUMAN ISLET AMYLOID POLYPEPTIDE
ZFP 407	-	ZINC FINGER PROTEIN 407
VEGF	-	VASCULAR ENDOTHELIAL GROWTH FACTOR

TABLE OF CONTENTS

INTRODUCTION

Diabetes mellitus refers to a group of common metabolic disorders that share the phenotype of hyperglycemia .The prevalence of type 2 diabetes mellitus is increasing rapidly accounting for 90-95 percent of the total diabetic population

Diabetes leads to a number of potentially disabling macro- and micro-vascular complications. Microvascular complications of diabetes include diabetic retinopathy (DR), diabetic nephropathy (DN), and diabetic peripheral neuropathy (DPN). Zinc plays a important role in both type 1 and type 2 diabetes (T2D). Serum zinc level is associated with Type 2 diabetes mellitus, and loss-of-function mutations in zinc transporter-8 gene protect against Type 2 Diabetes mellitus[1,2].

The development of microvascular complications in diabetes is majorly due to oxidative stress. Zinc has an antioxidative effect .Also it is a key component of many antioxidases. Lipid peroxidation induced damage is inhibited by zinc.Zinc induces the clearance of free radicals.[3] This suggest that zinc deficiency may be associated with the development of microvascular complications in diabetes mellitus

AIM & OBJECTIVES

1) AIM OF THE STUDY

The purpose of this study was to analyze the relationship between zinc level and each diabetic microvascular complication and identify the features related to low serum zinc level.

2) OBJECTIVES OF THE STUDY

To assess the serum zinc levels in subjects with type 2 diabetes mellitus.

To compare zinc levels in each microvascular complications.

To correlate between the zinc levels and microvascular complications.

REVIEW OF LITERATURE

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. There are several distinct types of diabetes mellitus which are caused by number of factors like genetic and environmental factors .

CLASSIFICATION

DM is classified on the basis of the pathogenic process that leads to hyperglycemia, as opposed to earlier criteria such as age of onset or type of therapy. There are two broad types of DM, designated type 1 and type 2.

Type 1 Diabetes Mellitus is the result of complete or near-total insulin deficiency. Although type 1 DM most commonly develops before the age of 30, an autoimmune beta cell destructive process can develop at any age. It is estimated that between 5 and 10% of individuals who develop Diabetes mellitus after age 30 years have type 1 Diabetes Mellitus [4].

Type 2 Diabetes Mellitus is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production. Distinct genetic and metabolic defects in insulin action and/or secretion are identified in type

2 DM[4]. Although type 2 DM more typically develops with increasing age, it is now being diagnosed more frequently in children and young adults,particularly in obese adolescents.Type 2 DM is preceded by impaired fasting glucose (IFG) or impaired glucose tolerance (IGT)

OTHER TYPES OF DM

Other etiologies for DM include specific genetic defects in insulin secretion or action, metabolic abnormalities that impair insulin secretion, mitochondrial abnormalities, and a host of conditions that impair glucose tolerance.

Maturity-onset diabetes of the young (MODY) and *monogenic diabetes* are subtypes of diabetes mellitus with an autosomal dominant inheritance, early age of onset (usually <25 years;), and impaired insulin secretion.

Pancreatic exocrine disease leads to DM when the majority of pancreatic islets are destroyed. Eg. Cystic fibrosis–related DM. Hormones antagonizing insulin action also lead to DM. And so DM is often a feature of endocrinopathies such as acromegaly and Cushing's disease. Viral infections also cause pancreatic islet destruction but are an extremely rare cause of DM. A form of acute onset of type 1 diabetes, termed *fulminant diabetes*, has been noted in Japan and may be related to viral infection of islet[4]

Chart 1 - Etiologic classification of diabetes mellitus

I. Type 1 diabetes
A. Immunologically mediated
B. Idiopathic
II. Type 2 diabetes
III. Other specific types
Genetic disorder of β-cell function (MODY, mitochondrial DNA)
Genetic disorders in insulin action (lipoatrophic diabetes)
Exocrine pancreas diseases (pancreatitis, hemochromatosis)
Endocrinopathies (acromegaly, Cushing's syndrom)
Drug-induced (*glucocorticoids*, tiazidics)
Infections (cytomegalovirus, congenital rubeola)
Uncommon immunological forms (insulin receptor antibodies)
Other genetic syndrome (Down, Turner, Prader-Willi syndrom)
IV. Gestational diabetes

Source: adapted from American Diabetes Association[9].

EPIDEMIOLOGY

Diabetes mellitus is the ninth major cause of death. About 1 in 11 adults worldwide now have DM. Asia accounts for a major proportion of the rapidly emerging Type 2 Diabetes mellitus global epidemic. China and India tops the list..Global burden of diabetes is more than 425 million people.with one third of people older than 65 years and 82 million people in south east asia and 72 million in India.This number is expected to rise to 629 million by 2045. The incidence of diabetes is on a increasing trend due to lifestyle changes and food habits.

There is much geographic variation in the incidence of diabetes mellitus. The prevalence also varies among different ethnic populations.The developing regions of the world like the African, Asian, and South American regions account for 77 percentage of diabetics[5,6,7] The prevalence of DM is similar in both sexes. Diabetes accounts for one in four health care dollars in the U.S. Diabetics , on average, have expenditures ~2.3 times higher than expenditures of non diabetics.

Criteria for the Diagnosis of Diabetes Mellitus[9]

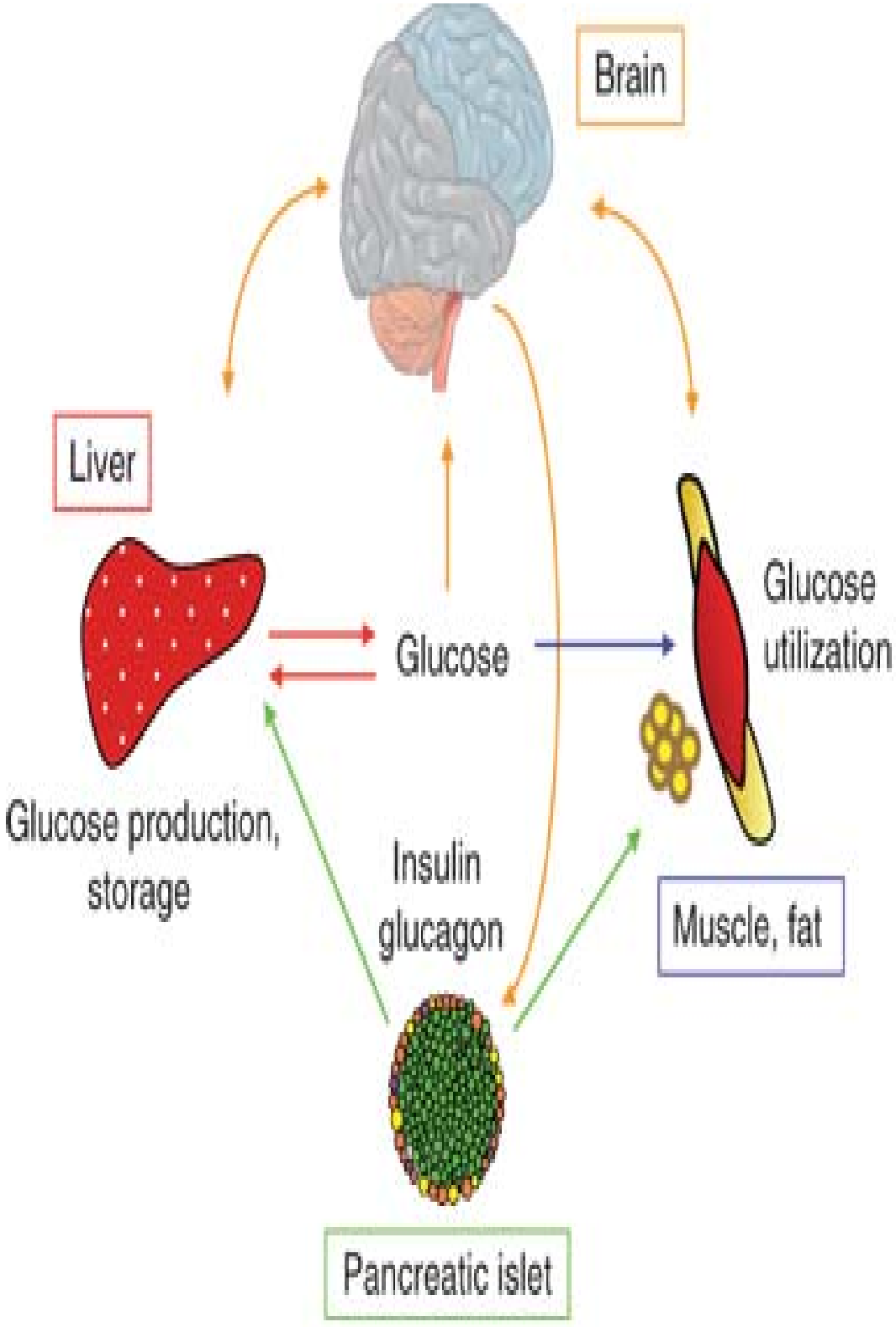
- Symptoms of diabetes plus random blood glucose concentration ≥11.1 mmol/L (200 mg/dL) *or*
- Fasting plasma glucose ≥7.0 mmol/L (126 mg/dL) *or*

- Hemoglobin A1c ≥ 6.5%*c or*
- 2-h plasma glucose ≥11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test

REGULATION OF GLUCOSE HOMEOSTASIS

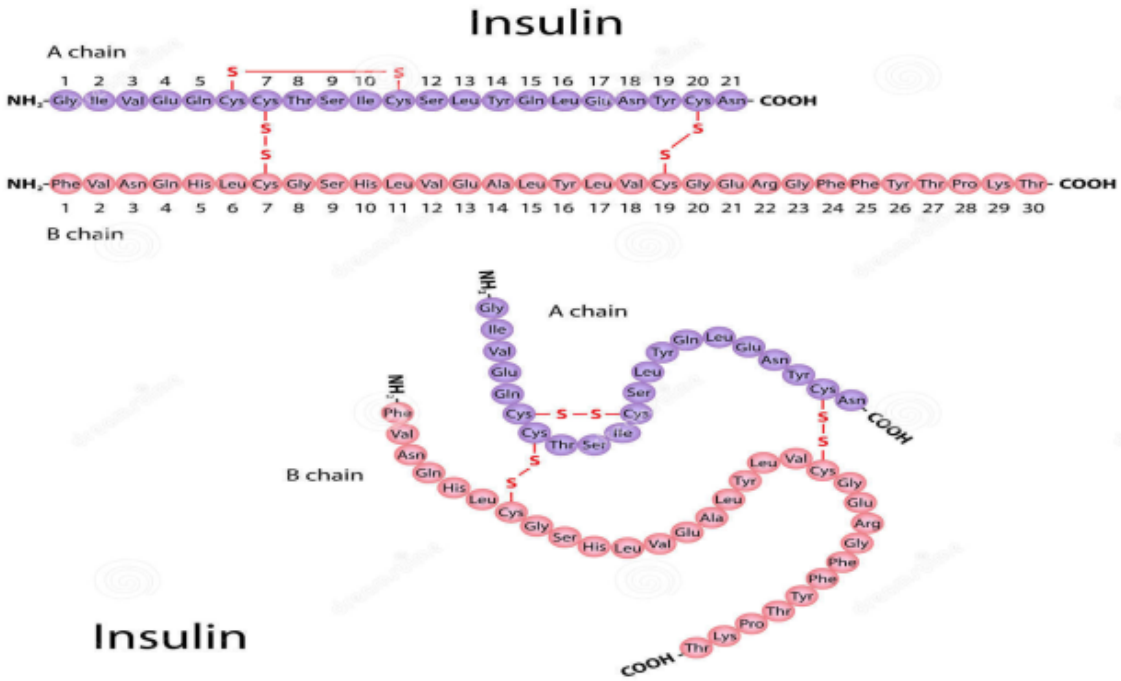
Glucose homeostasis is a balance between hepatic gluconeogenesis and peripheral glucose uptake and utilization. The most important regulator of this equilibrium is insulin. Other regulators are neural mechanisms , metabolic signals and various hormones (e.g., glucagon) . Glucose homeostasis is also influenced by factors like leptin, adiponectin, resistin, irisin etc.

Regulation of glucose homeostasis. The organs shown contribute to glucose utilization, production, or storage.



INSULIN BIOSYNTHESIS

The beta cells of the pancreatic islets secrete insulin. Insulin is synthesized initially as preproinsulin.. Preproinsulin is a eighty six – aminoacid polypeptide. Proteolytic processing of this poly peptide give rise to proinsulin which generates the C peptide and the A (21amino acids) and B (30 amino acids) chains of insulin.. The mature insulin molecule and C peptide are stored together and co-secreted from secretory granules in the beta cells.



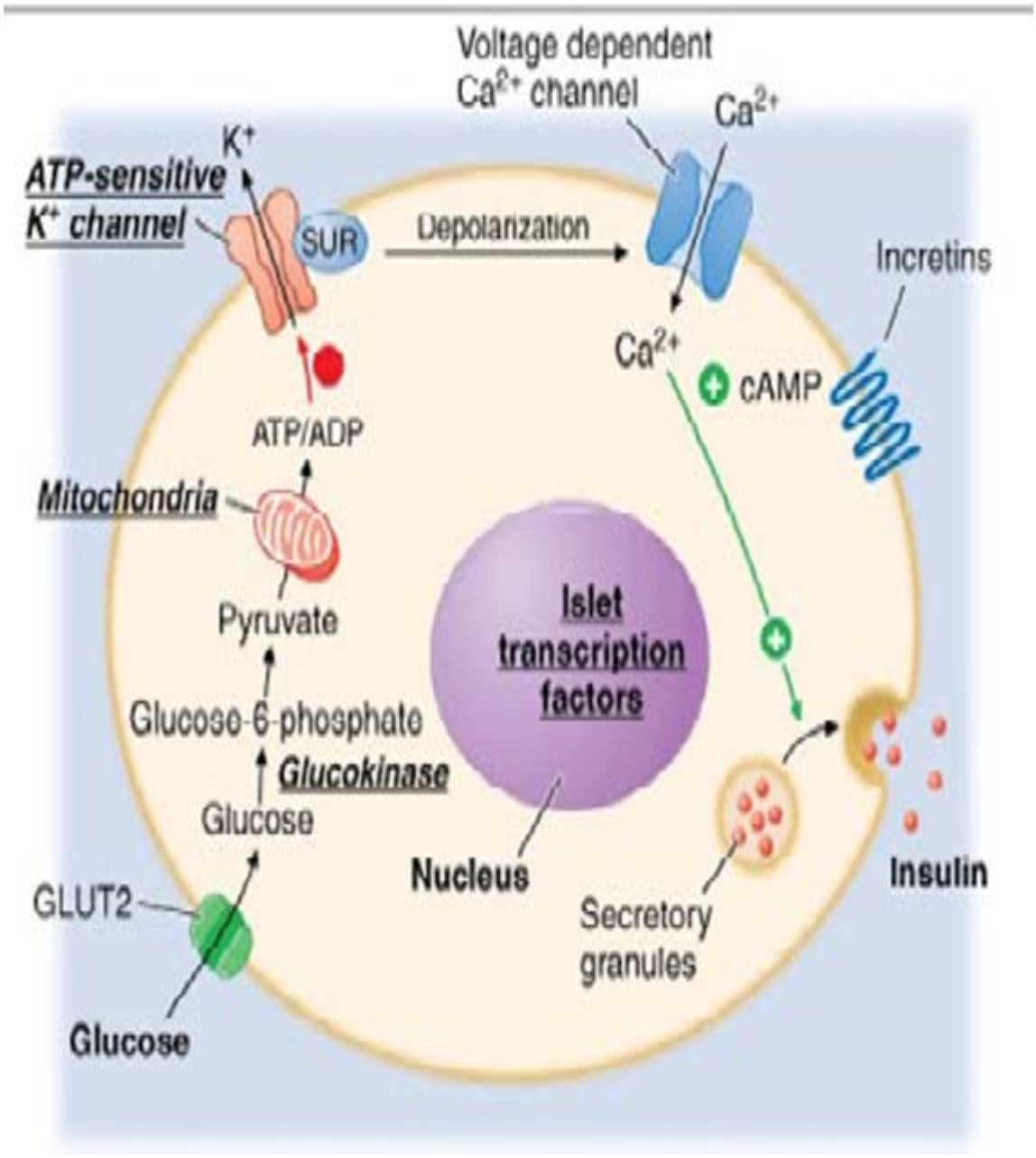
INSULIN SECRETION

The key regulator of insulin secretion by the pancreatic beta cell is insulin. Insulin secretion is also influenced by amino acids, ketones, various nutrients, gastrointestinal peptides, and neurotransmitters. Insulin synthesis is stimulated when glucose levels are greater than 70mg/dl , primarily by enhancing protein translation and processing.

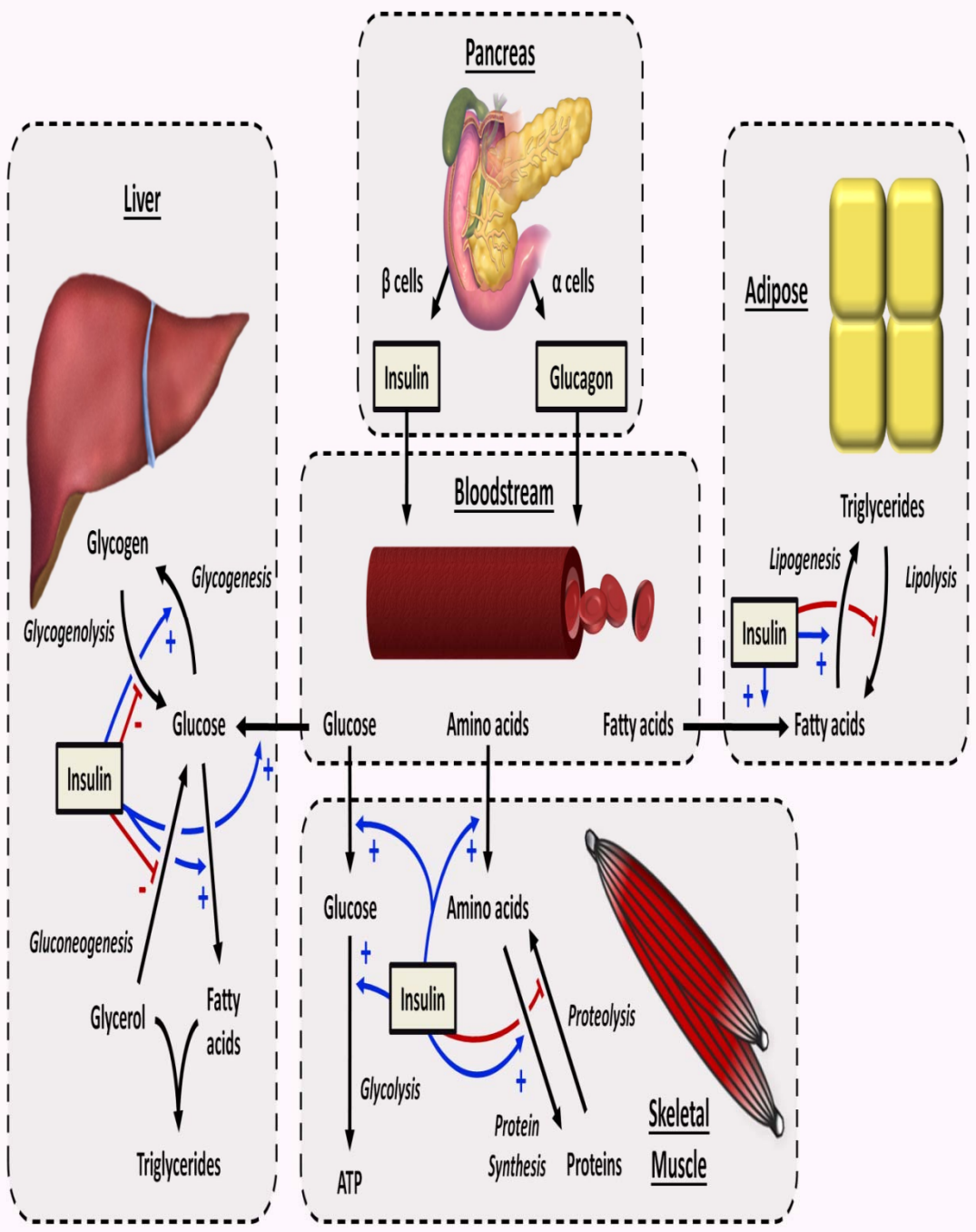
The process of insulin secretion starts with glucose entry into the beta cell. Glucose is transported into the beta cell by a facilitative glucose transporter. After which glucose phosphorylation by glucokinase occur. Metabolism of glucose-6-phosphate subsequently, through glycolysis produces ATP. This inhibits the activity of an ATP-sensitive K+ channel. Inhibition of potassium channel produces beta cell membrane depolarization, which opens voltage-dependent calcium channels and leads to insulin secretion[4].

There is a pulsatile pattern of hormone release, with small secretory bursts occurring about every 10 min, superimposed upon greater amplitude oscillations of about 80–150 min. Incretins are released from neuroendocrine cells of the gastrointestinal tract following food ingestion and amplify glucose-stimulated insulin secretion and suppress glucagon secretion.

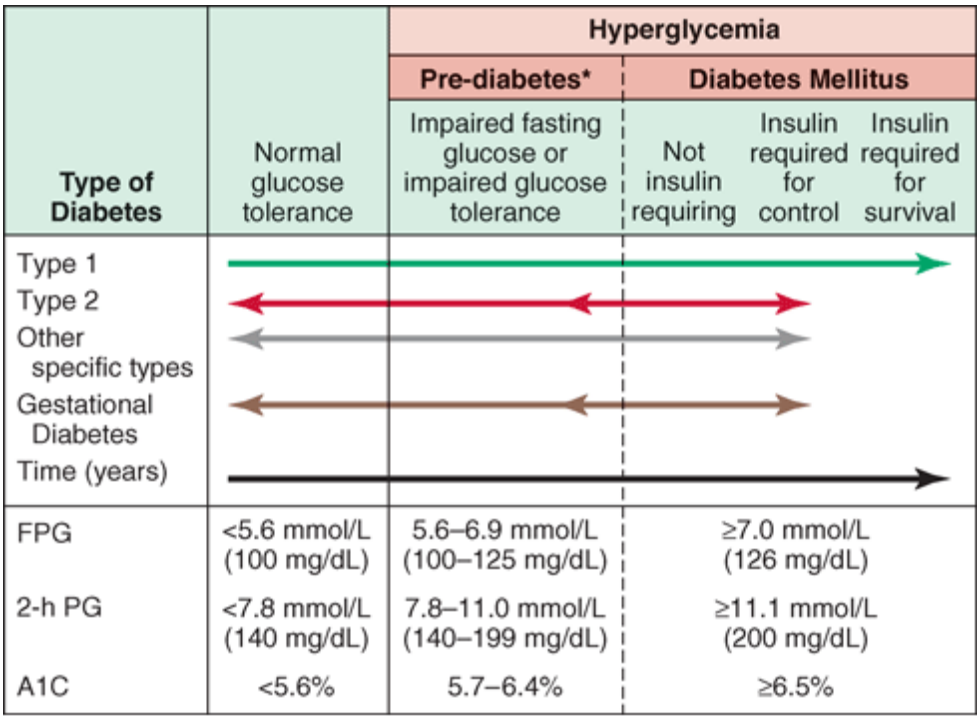
Mechanisms of glucose-stimulated insulin secretion



Actions of Insulin



Spectrum of glucose homeostasis and DM



Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

PATHOGENESIS

TYPE 2 DIABETES MELLITUS

Insulin resistance and abnormal insulin secretion leads to the development of type 2 DM. Although the primary defect is controversial,most studies support the view that insulin resistance precedes an insulin secretory defect but that diabetes develops only when insulin secretion becomes inadequate[4].

GENETIC CONSIDERATIONS

Type 2 DM has a strong genetic component. The concordance of type 2 DM in identical twins is between 70 and 90% [4]. Individuals with a

parent with type 2 DM have an increased risk of diabetes. if both parents have type 2 DM, the risk approaches 40%.

Insulin resistance, as demonstrated by reduced glucose utilization in skeletal muscle, is present in many nondiabetic, first-degree relatives of individuals with type 2 DM. Genetic polymorphisms have also been found in the genes encoding the PPARγ, K^+ channel , Insulin receptor substrates etc.[4]

Pathophysiology

Type 2 Diabetes mellitus has impaired insulin secretion, insulin resistance, excessive gluconeogenesis in liver, and abnormalities in lipid metabolism. Obesity, particularly visceral or central , is a very common accompainement of type 2 DM. In the early stages of the disorder, inspite of insulin resistance glucose tolerance remains normal , because the pancreatic beta cells compensate by increasing insulin output.

As insulin resistance and compensatory hyperinsulinemia progress, the pancreatic islets are not able to withstand the hyperinsulinemic state. IGT, characterized by elevations in postprandial glucose, then develops. A further decrease in secretion of insulin and an increase in hepatic gluconeogenesis lead to overt diabetes with fasting hyperglycemia. Ultimately, beta cell failure ensues.

Metabolic Abnormalities

Abnormal muscle and fat metabolism

Insulin resistance, the decreased ability of insulin to act on target tissue is a marked feature of type 2 Diabetes mellitus .This results from a combination of genetic susceptibility and obesity. Insulin resistance affects glucose utilization by insulin-sensitive tissues and increases hepatic glucose output. Both effects contribute to the hyperglycemia.

Increased hepatic glucose output predominantly accounts for increased FPG levels, whereas decreased peripheral glucose usage results in postprandial hyperglycemia.

IMPAIRED INSULIN SECRETION

Insulin secretion and sensitivity are interrelated. In type 2 DM, insulin secretion initially increases in response to insulin resistance to maintain normal glucose tolerance. Initially, the insulin secretory defect is mild and selectively involves glucose-stimulated insulin secretion, including a greatly reduced first secretory phase. The response to other nonglucose secretagogues, such as arginine, is preserved, but overall beta function is reduced by as much as 50% at the onset of type 2 Diabetes Mellitus.

INCREASED HEPATIC GLUCOSE AND LIPID PRODUCTION

In type 2 DM, insulin resistance in the liver reflects the failure of hyperinsulinemia to suppress gluconeogenesis, which results in fasting hyperglycemia and decreased glycogen storage by the liver in the postprandial state. Increased hepatic glucose production occurs early in the course of diabetes, although likely after the onset of insulin secretory abnormalities and insulin resistance in skeletal muscle. As a result of insulin resistance in adipose tissue, lipolysis and free fatty acid flux from adipocytes are increased, leading to increased lipid lipoprotein synthesis in hepatocytes.

Insulin Resistance Syndromes

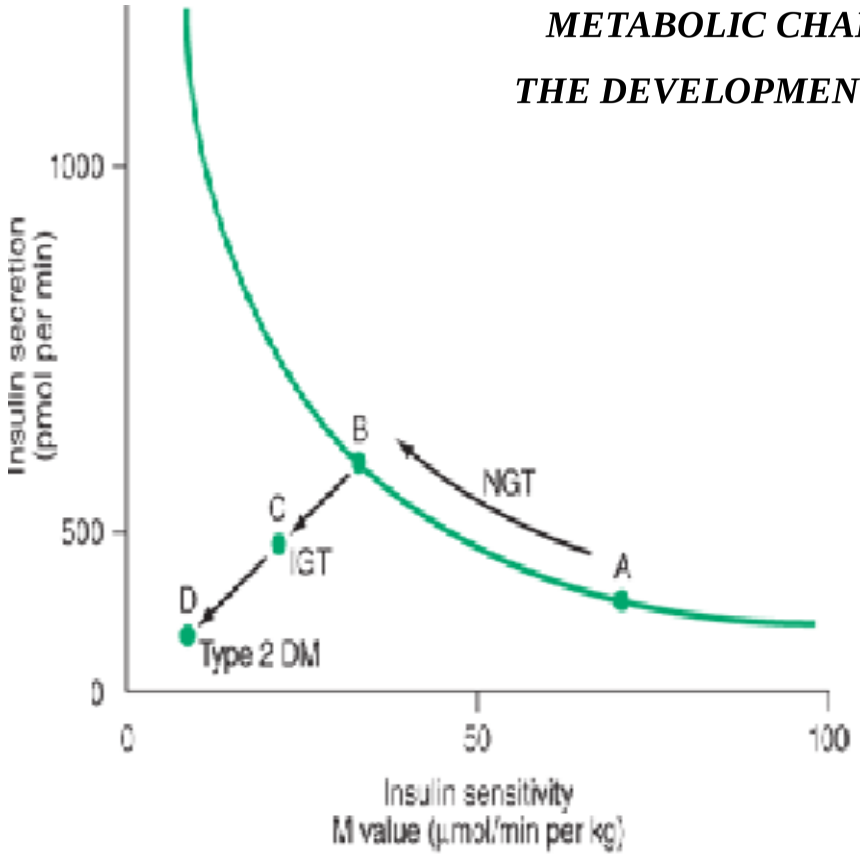
The insulin resistance condition comprises a spectrum of disorders, with hyperglycemia representing one of the most readily diagnosed features. The *metabolic syndrome*, the *insulin resistance syndrome*, and *syndrome X* are terms used to describe a constellation of metabolic derangements that includes insulin resistance, hypertension, dyslipidemia, central or visceral obesity, type 2 DM or IGT/IFG,

Mutations in the insulin receptor that interfere with binding or signal transduction are a rare cause of insulin resistance. Acanthosis nigricans and signs of hyperandrogenism (hirsutism, acne, and oligomenorrhea in women) are also common physical features. Polycystic ovary syndrome (PCOS) is a common disorder that affects premenopausal women and is characterized by chronic anovulation and hyperandrogenism. Insulin resistance is seen in a significant subset of women with PCOS, and the disorder substantially increases the risk for type 2 DM, independent of the effects of obesity.

Two distinct syndromes of severe insulin resistance have been described in adults: (1) type A, characterized by severe hyperinsulinemia, obesity, and features of hyperandrogenism; and (2) type B characterized by severe hyperinsulinemia, features of hyperandrogenism, and autoimmune disorders

METABOLIC CHANGES DURING

THE DEVELOPMENT OF TYPE 2 DM



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www.accessmedicine.com

Risk factors for type 2 Diabetes mellitus

- Family history of diabetes (i.e., parent or sibling with type 2 diabetes)
- Obesity (BMI >25 kg/m^2)
- Habitual physical inactivity
- Race/ethnicity (e.g., African American, Latino, Native American, Asian American, Pacific Islander)
- Previously identified IFG or IGT
- History of GDM or delivery of baby >4 kg (>9 lb)
- Hypertension (blood pressure >140/90 mmHg)
- HDL cholesterol level <35 mg/dL (0.90 mmol/L) and/or a triglyceride level >250 mg/dL (2.82 mmol/L)
- Polycystic ovary syndrome or acanthosis nigricans
- History of vascular disease

ROLE OF ZINC IN DIABETES AND IN MICROVASCULAR COMPLICATIONS

Zinc plays a major role in the development of both type 1 and type 2 diabetes (T2D). Studies have shown that serum zinc level is associated with T2D, and loss-of-function mutations in zinc transporter-8 gene protect against T2D.

Zinc has a number of useful effects in both types of diabetes mellitus. [(1O-13)]. This has been shown in many *In-vitro* and *in-vivo* studies in animals and humans. Decreased serum zinc levels and increased urinary excretion is known to be present in patients with both types of diabetes mellitus [15,16] .

TBARS is a marker of oxidative stress. Levels are high diabetic patients, and are reduced by zinc supplementation.[11,15] Se-GPx was low in patients with type-1 diabetes. With supplementation of zinc ,levels of Se-GPx returned to normal levels[11]. Antioxidant enzymes like catalase, GPx and super-oxide dismutase (SOD) are reduced in diabetes induced animal models. [17-19]

Supplementation of zinc in these animals increased the enzyme activity[17-19]. Metallothionein (MT) protein (an antioxidant) synthesis in

the pancreatic islets, kidneys, liver and heart of diabetes-induced animals ,significantly increased after supplementation of zinc. [19,20,21-2]

Zinc supplementation reduces renal oxidative damage and also downregulates expression of pro-fibrosis mediators in diabetics mediated via the expression of MT[25].

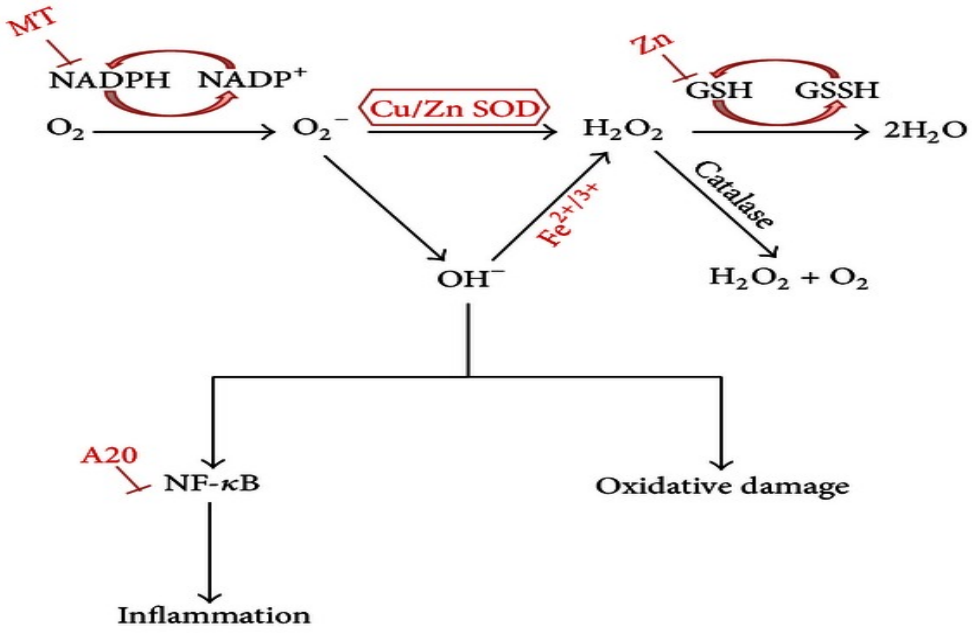
MDA levels is an index of lipid peroxidation. It was increased in diabetic animals. [18,20] Supplementation of zinc markedly reduced MDA levels.Zinc supplementation increases insulin sensitivity and action of antioxidants. [11,26]

Rats simultaneously treated with a single injection of alloxan and Zinc chloride showed that hyperglycemia induced by alloxan was significantly reduced at 24, 48, and 72 h post-treatment with Zinc chloride[27]. ZnCl injection also accentuated glutathione in retina, pancreas and liver.

Zinc supplementation increases TNF-α gene expression in post menopausal diabetics. This suggests a interaction between Zinc homeostasis and oxidative stress[28]. MT synthesis is increased by zinc supplementation and therby has a beneficial effect in diabetic neuropathy by reducing oxidative stress [29].

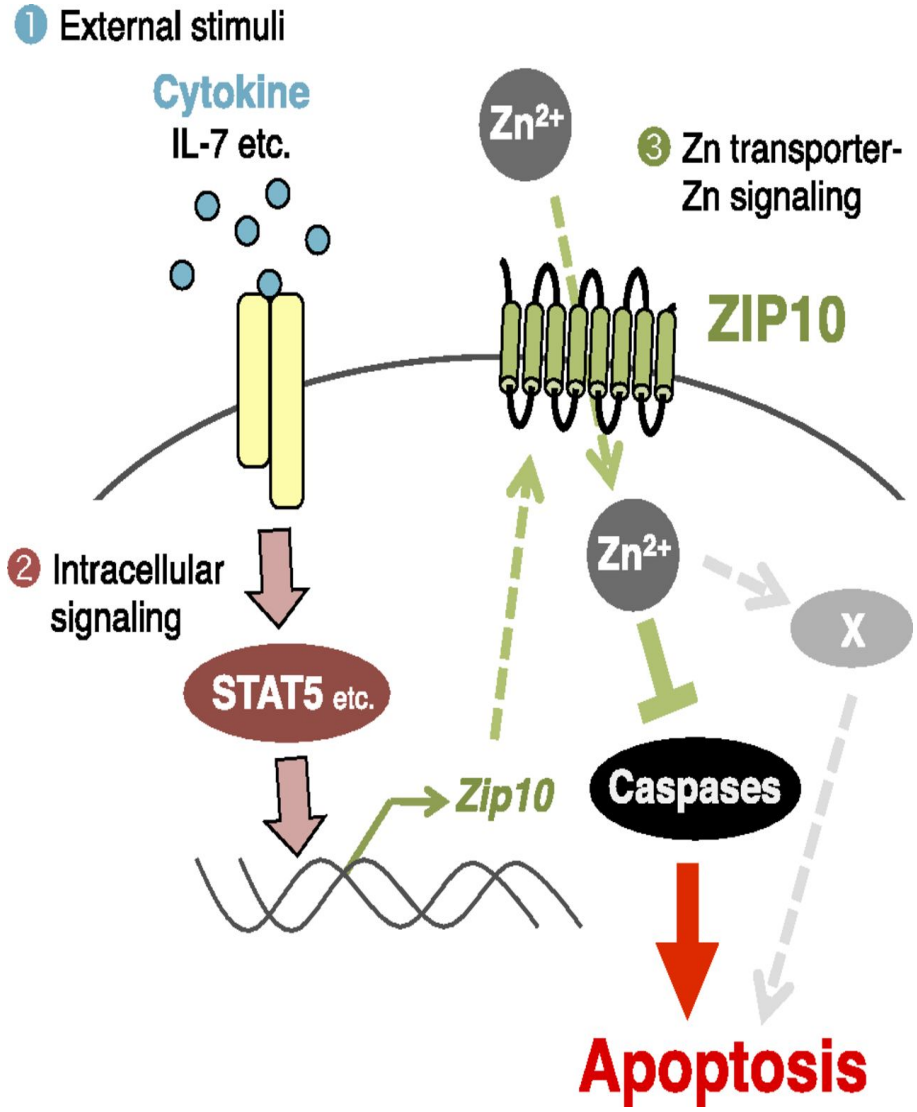
Zinc supplementation inhibited hyperglycemia induce cell death by decreasing reactive oxygen species production, facilitated via Nrf2 up-regulation in renal tubular epithelial cells. [30].Studies reveal that diabetes is commonly accompanied by decrease in serum zinc levels and increased zinc excretion.[31,32]

Zinc supplementation improves glycemic control and promotes healthy lipid parameters [33].



Proposed mechanism of zinc effect on oxidative stress and inflammation. Zinc attenuates oxidative damage and inflammation via MT, Cu/Zn SOD, Zn-finger protein, and itself

Zinc transporter facilitates anti-apoptotic signaling



α-glucosidase activity in the intestines is inhibited by zinc. [35,36]. In skeletal muscles Zinc-α2-glycoprotein stimulates the phosphorylation of AMP-activated protein kinase (AMPKα) and increases cellular GLUT4 protein[37]. This increase in the expression of the GLUT4 has also been observed in adipose tissue with resultant increase in glucose uptake [38,39].

Zinc Finger Protein 407 (ZFP407) is known to facilitate insulin stimulated glucose uptake via glucose transporter-4.[40,41]. Zinc also increased glucose transport in adipocytes, independent of insulin[42-44].

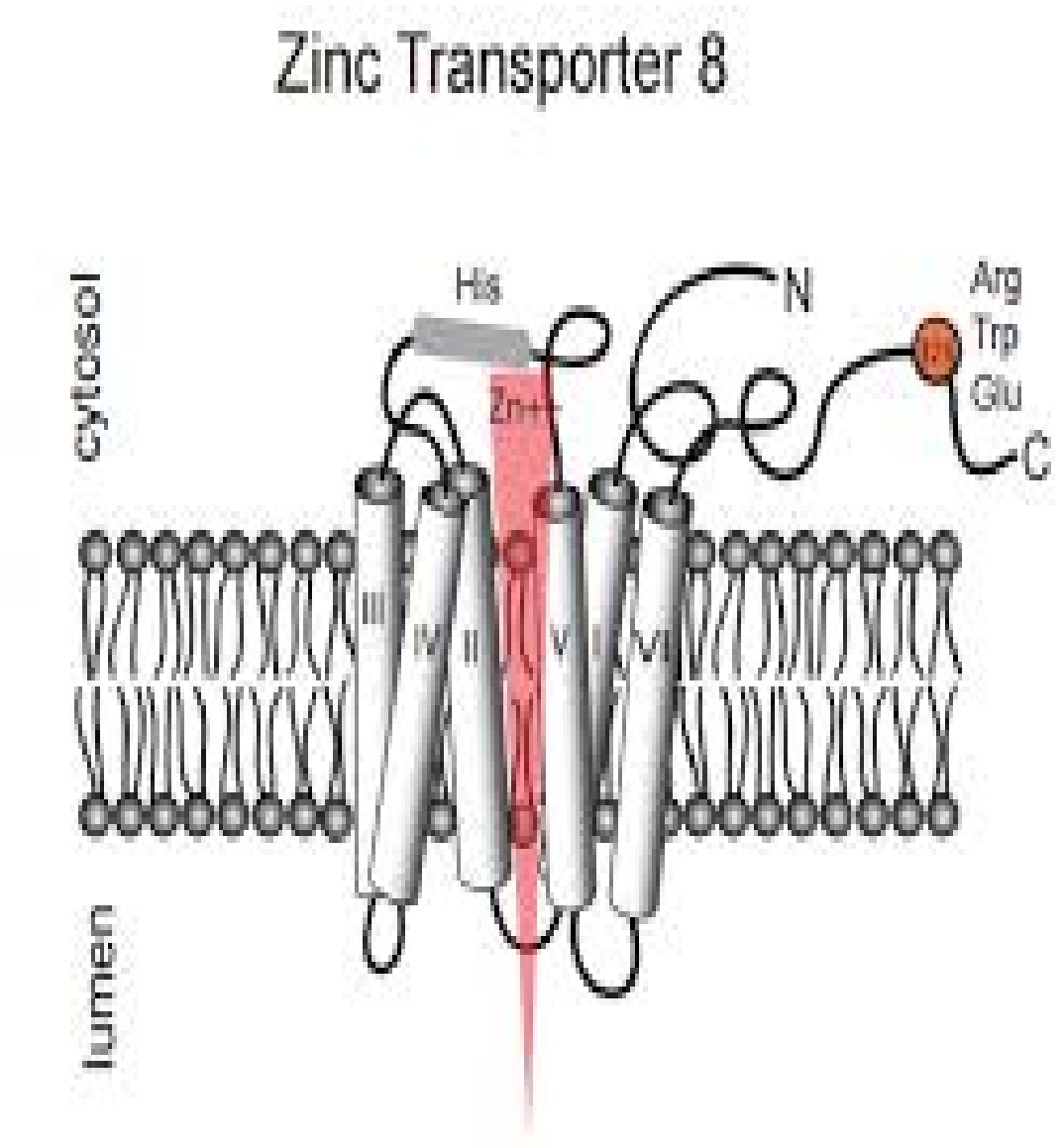
Zinc stimulates the phosphorylation of the InsulinReceptor beta subunit[42]. Zinc also inhibits Glycogen synthase kinase and therby increases glycogen synthesis[43].

In human pancreatic islet cells, Zinc transporter 8 is the key protein which plays a major role in both Zinc accumulation in insulin-containing vesicles and regulation of insulin secretion[45-49]. ZnO nanoparticles at dose 70ng/ml reduced oxidative stress and improved pancreatic function [50.] Intracellular Zinc inhibits glucagon secretion [51].

Human Islet Amyloid Polypeptide (hIAPP) (a polypeptide hormone secreted from pancreatic β-cells in response to glucose) and is cleared by the peptidases in the kidney. hIAPP is known to aggregate in the pancreas to form dense, insoluble extracellular fibrillar deposit, causing β-cell destruction in type-2 diabetes

Zinc, significantly inhibits hIAPP amyloid fibrillogenesis at concentrations similar to those found *in-vivo* extracellular environments . This probably explains the linkage between the mutations of SLC30A8 zinc transporter (Zinc Transporter 8 [ZnT8]), which transports Zinc into the secretory granules, and type-2 diabetes.

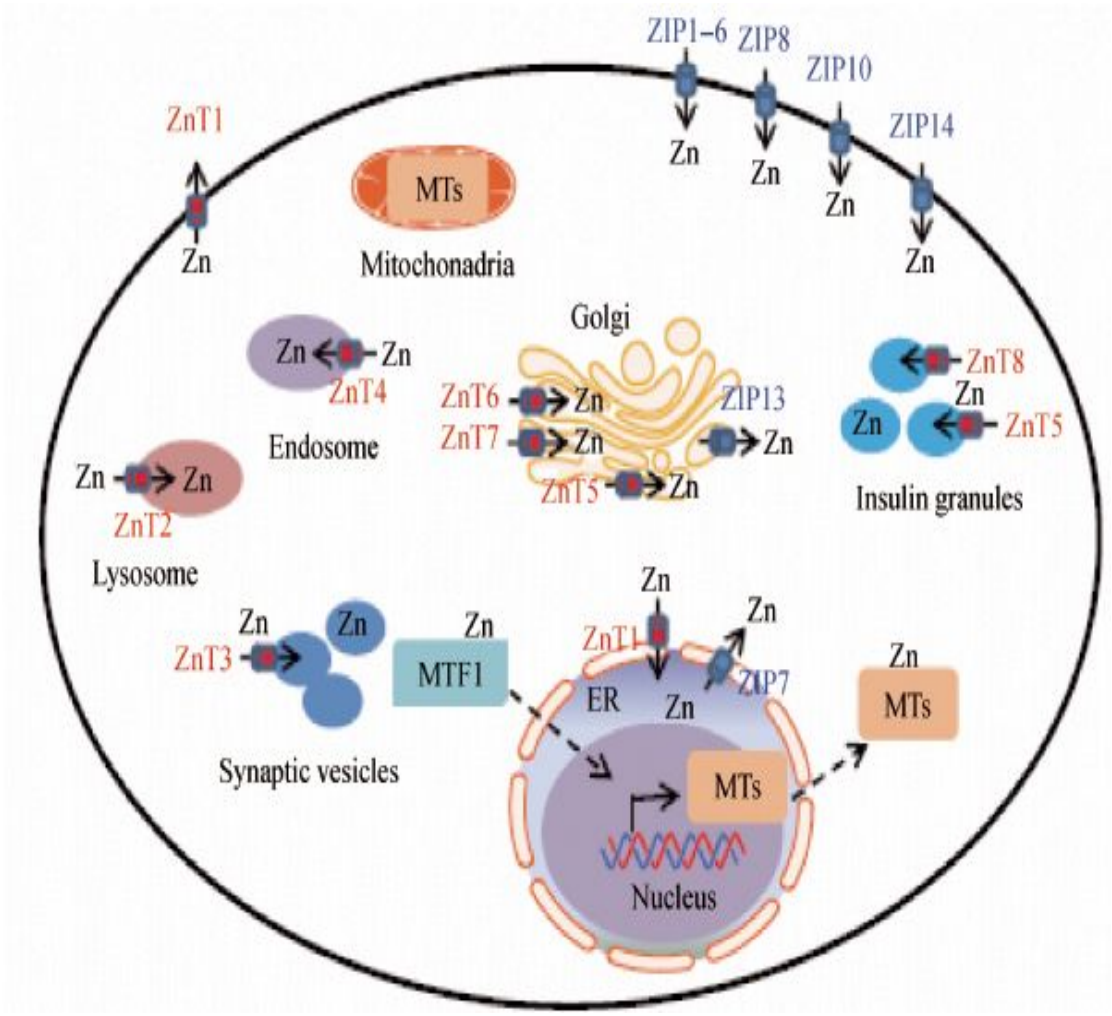
When ZnT8 absent mice were fed a control diet, glucose tolerance and insulin sensitivity were normal. However, after high-fat diet feeding, these mice became glucose intolerant or diabetic, and islets became less responsive to glucose. ZnT8 is downregulated on exposure to metabolic stress associated with diabetic and pre-diabetic states, suggesting that it might further contribute to progression of type-2 diabetes.



In β-cell specific SLC30A8 deficiency (ZnT8 knockout mice) a low peripheral blood insulin levels was observed, due to a substantial amount of the insulin being degraded during its first passage through the liver.

This is possibly due to the low level of Zinc in the portal circulation co-secreted by β-cells, due to the absence of ZnT8 (reducing uptake of Zinc by β-cells), which leads to augmented hepatic insulin

clearance. The ZnT8 is also downregulated in response to exposure of pancreatic β-cells to hypoxia, resulting in lowered cytosolic Zn concentrations.



Pancreatic islet cells harvested from rats conditioned under intermittent hypoxia showed a significant reduction in Zinc Influx Transporter 8 (ZIP8) expression in the β-cell membrane, with resultant reduction in cellular Zinc concentration and insulin production

ZIP6 and ZIP7 function as two important zinc influx transporters to regulate cytosolic Zinc concentrations and insulin secretion in β-cells and ZIP-6 is also capable of directly interacting with GLP-1R to facilitate the protective effect of GLP-1 on β-cell survival .

Zip4 protein is located in human pancreaticβ-cells, is important for the accumulation of Zinc in the cytosol and granules of β-cells . Other Zinc transporters like ZnT3 and ZIP7 might also play a role in insulin secretion and glucose metabolism

L-type voltage-gated Ca channels and TRMP3 (transient receptor potential cation channel subfamily M member 3) are also in part responsible for Zinc transport into β-cells, which is also dependent upon the metabolic status of the cell . Culture of rat pancreatic islets in either low or high vs. intermediate glucose concentrations triggers early mitochondrial oxidative stress and late β-cell apoptosis with loss of glucose stimulated insulin secretion.

It is well known that Reactive Oxygen Species (ROS) can cause pancreatic β-cell death. This occurs due to the activation of Transient Receptor Potential Melastatin2 (TRPM2) channels by ROS. TRPM2 causes Ca influx into the β-cells causing release of lysosomal Zinc, which results in β-cell death

In glucagon producing α-cells of the pancreas Zinc accumulates under low and high glucose conditions through both Ca channels and other Zinc transporting mechanisms, and the intracellular Zinc inhibits glucagon secretion . Furthermore during hypoglycemia the principal signal that initiates glucagon secretion could be the detection by α-cells of a sudden decrease in Zinc paralleling the fall in insulin in the islet peri-portal circulation and this drop in concentration of Zinc, closes α-cell ion channels, promoting entry of calcium which stimulates glucagon secretion

Zinc-α2-glycoprotein is gaining increasing recognition as a marker of insulin resistance in type-2 diabetes. Zinc-α2-glycoproteins are also involved in lipid metabolism, affecting the expression of several lipolytic enzymes at hepatic and adipose tissue level. Zinc supplementation reduces Fasting Blood Glucose, 2 h Post Prandial Blood Glucose and HbA1c in patients with diabetes, as well as reducing total cholesterol, LDL cholesterol and triglycerides in both patients with and without

diabetes]. The above molecular/enzymatic level mechanisms probably explain the beneficial effects of Zinc supplementation on glycaemic control and lipids observed in humans

Zinc supplementation increases the activity and levels of key antioxidant enzymes and proteins, while lowering lipid peroxidation. Oxidative stress also plays a important role in the pathogenesis of both micro- and macro-vascular complications of diabetes [52].. Increased formation of AGEs leads to diabetes related complications [52]. Zinc supplementation markedly inhibited the formation of advanced glycosylation end products.

COMPLICATIONS OF DIABETES MELLITUS

Diabetes leads to varied complications which affect many organ systems and majority of morbidity and mortality associated with the disease is attributed to its complications. Strikingly, in the United States, diabetes is the leading cause of new blindness in adults, renal failure, and non traumatic lower extremity amputation[4].

Diabetes-related complications usually appear after the second decade of hyperglycemia. Many individuals with type 2 DM have complications at the time of diagnosis because of the long asymptomatic period of increased blood sugars.

Diabetes-related complications can be divided into vascular and nonvascular complications.

The diabetic vascular complications are microvascular (retinopathy, neuropathy, nephropathy) and macrovascular complications (coronary heart disease [CHD], peripheral arterial disease [PAD], cerebrovascular disease). Microvascular complications are diabetes-specific. Macrovascular complications are similar to those in nondiabetics but occur at greater frequency in individuals with diabetes[4]. Gastroparesis, infections, skin changes, and hearing loss are non vascular complications.

The risk of dementia or impaired cognitive function is whether increased by type 2 diabetes is not clear

Microvascular complications of DM include diabetic retinopathy, diabetic nephropathy and diabetic peripheral neuropathy.

DIABETIC RETINOPATHY

DM is the leading cause of blindness between the ages of 20 and 74 in the United States. Diabetics are 25 times more likely to become legally blind than individuals without DM. Progressive diabetic retinopathy and clinically significant macular edema leads to severe vision loss

Pathogenesis

Aldose reductase plays a important role in the development of diabetes complications. Aldose reductase is the initial enzyme in the intracellular polyol pathway, which involves the conversion of glucose into sorbitol. Hyperglycemia increase the flux of sugar molecules through the polyol pathway, leading to sorbitol accumulation in cells. sorbitol accumulation leads to osmotic stress mechanism which leads to diabetic retinopathy.[4]

Hyperglycemia can stimulate free radical production and reactive oxygen species formation leading to cellular injury

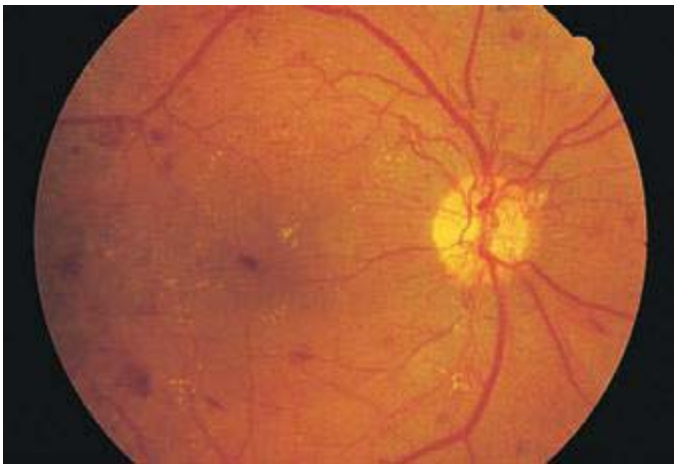
Growth factors, like vascular endothelial growth factor (VEGF), TGF β etc have been found to play major roles in the development of diabetic retinopathy[4]

Diabetic retinopathy is generally classified as either background or proliferative.Background retinopathy includes such features as small hemorrhages in the middle layers of the retina. They clinically appear as "dots" and therefore are frequently referred to as "dot hemorrhages." Hard exudates are caused by lipid deposition that typically occurs at the margins of hemorrhages

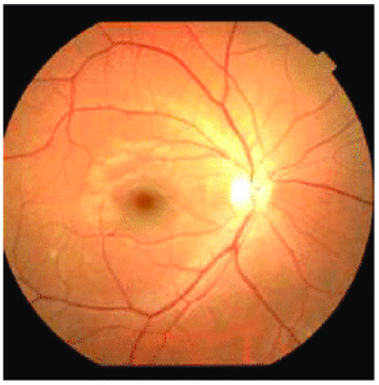
Microaneurysms are small vascular dilatations that occur in the retina, often as the first sign of retinopathy[4]. Retinal edema may result from microvascular leakage and is indicative of compromise of the blood-retinal barrier. The appearance of neovascularization in response to retinal hypoxemia is the hallmark of proliferative diabetic retinopathy[4].

These newly formed vessels appear near the optic nerve and/or maculaand rupture easily, leading to vitreous hemorrhage, fibrosis, and ultimately retinal detachment

Diabetic retinopathy with scattered hemorrhages, yellow exudates and neovascularization



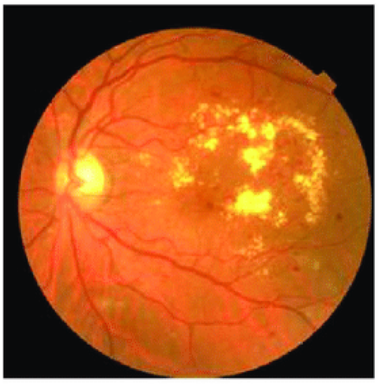
Category/description
Non-proliferative diabetic retinopathy (NPDR)
No DR
Very mild Microaneurysms only
Mild Any or all of: microaneurysms, retinal haemorrhages, exudates, cotton wool spots, up to the level of moderate NPDR. No IRMA or significant beading
Moderate · Severe retinal haemorrhages (more than ETDRS standard photograph 2A: about 20 medium-large per quadrant) in 1–3 quadrants *or* mild intraretinal microvascular abnormalities (IRMA) · Significant venous beading can be present in no more than 1 quadrant · Cotton wool spots commonly present
Severe The 4-2-1 rule; one or more of: · Severe haemorrhages in all 4 quadrants · Significant venous beading in 2 or more quadrants · Moderate IRMA in 1 or more quadrants
Very severe Two or more of the criteria for severe
Proliferative diabetic retinopathy (PDR)
Mild-moderate New vessels on the disc (NVD) or new vessels elsewhere (NVE), but extent insufficient to meet the high-risk criteria
High-risk · New vessels on the disc (NVD) greater than ETDRS standard photograph 10A (about ½ disc area) · Any NVD with vitreous or preretinal haemorrhage · NVE greater than ½ disc area with vitreous or preretinal haemorrhage (or haemorrhage with presumed obscured NVD/E)



Without DR

Early diabetic retinopathy

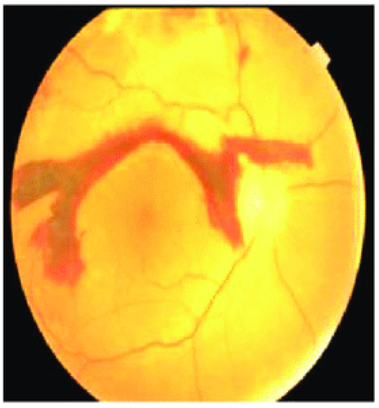
Mild NPDR



Moderate NPDR

Severe NPDR

PDR and neovascularization



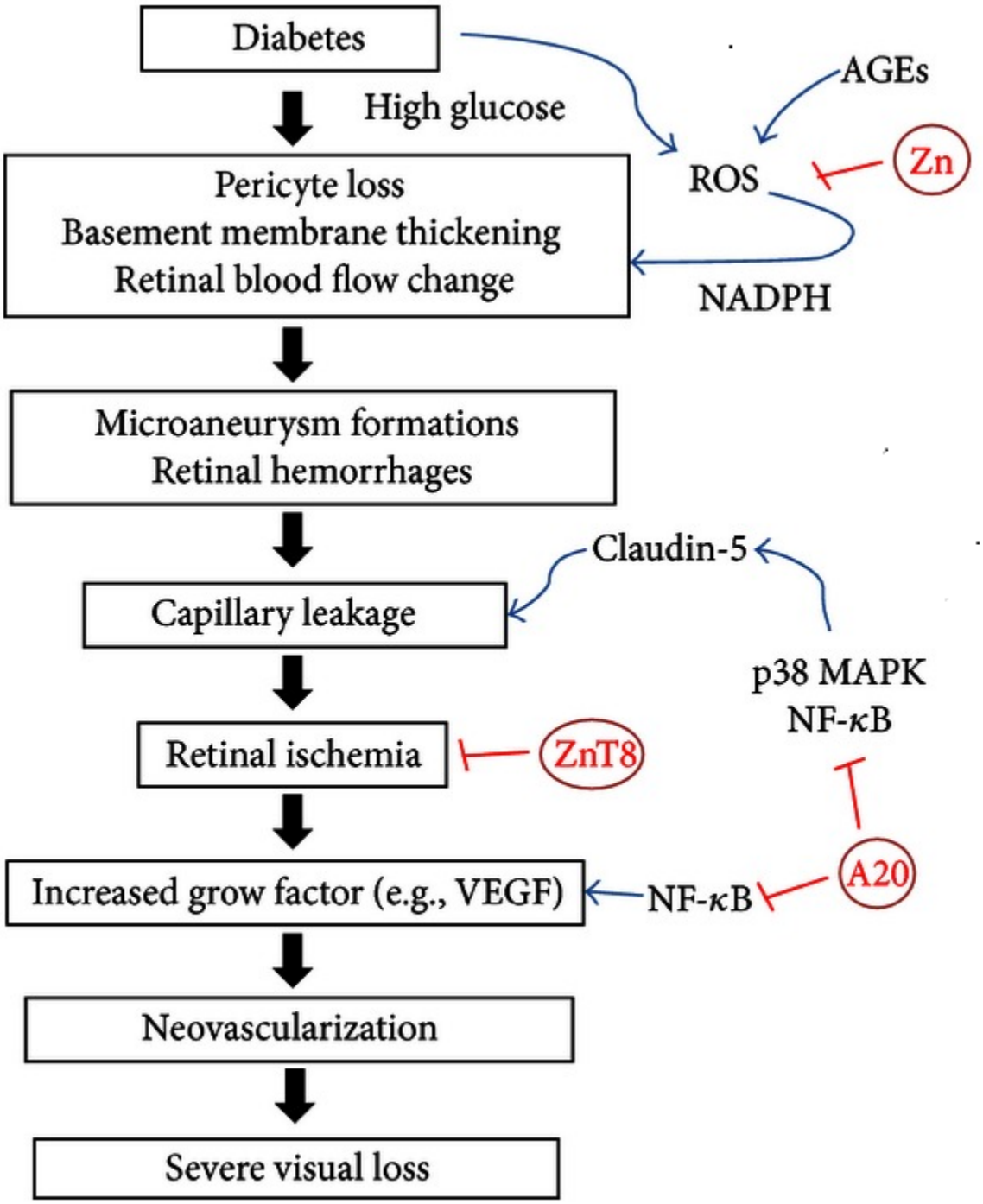
PDR with vitreous hemorrhage

PDR with vitreous hemorrhage and PLM

Vitreoretinal traction bands

It is well known that hyperglycemia accelerates the formation of advanced glycation end products (AGEs), which have been implicated in the pathogenesis of DR [53]. They can stimulate ROS production in retinal pericytes, largely via activation of NADPH oxidase, which results in retinal pericyte apoptosis [54]. It is suggested that Zn might prevent retinal pericyte apoptosis via inhibition of NADPH oxidase in DR..

Ocular neovascularization, which is most potently caused by hypoxia and ischemia, is also a key component in DR [55,56]. It has been convincingly demonstrated that hypoxia inducible factor-1 (HIF-1) and VEGF are involved in the initiation and progression of neovascularization in DR [57]. Zn reduces inflammatory cytokine production by upregulating the Zn-finger protein, A20, which inhibits NF-κB activation via the TRAF pathway [58]. In addition, recent finding suggests that ZnT8 expression was reduced by ischemic insults and to restore the ZnT8 to its basal homeostatic levels can prevent retinas from ischemia induced injury [59]

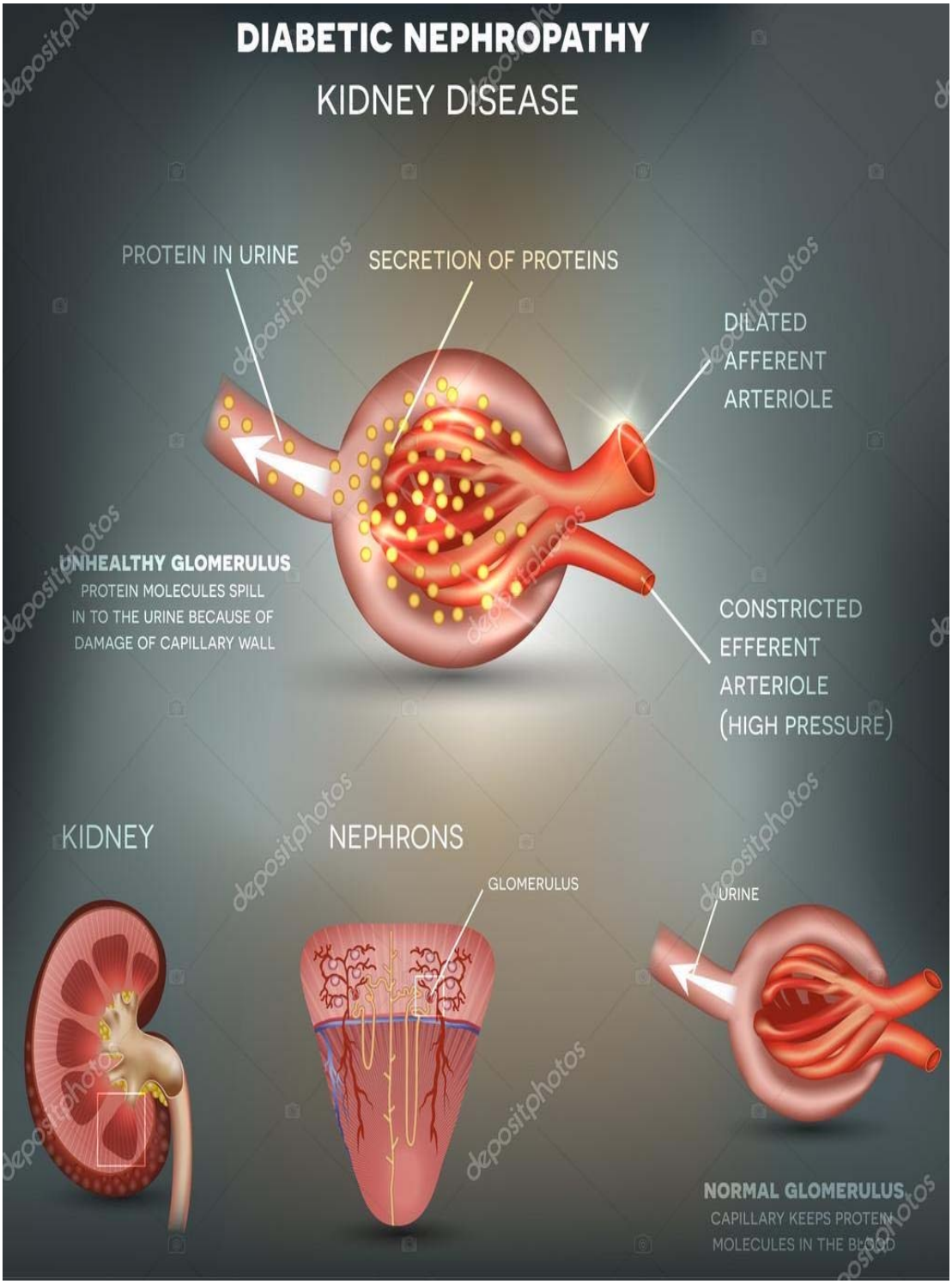


Proposed mechanism by which zinc protects from DR. Zinc protects DR bysuppressing the pericyte apoptosis, capillary leakage, and neovascularization.

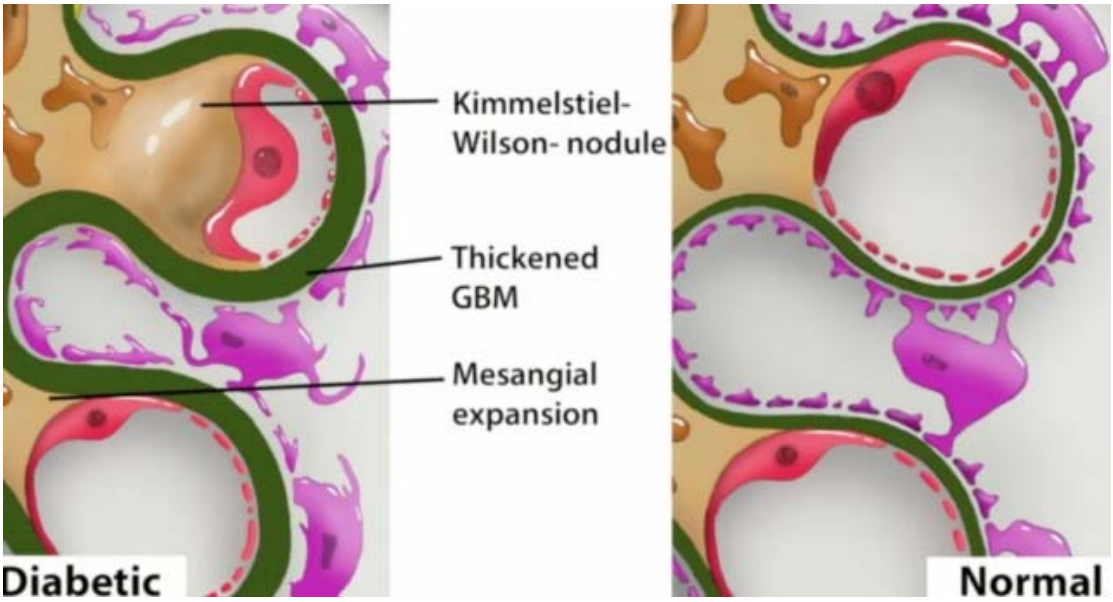
DIABETIC NEPHROPATHY

Diabetic nephropathy is the leading cause of chronic kidney disease (CKD), ESRD, and CKD requiring renal replacement therapy. Albuminuria in individuals with DM is associated with an increased risk of cardiovascular disease. Individuals with diabetic nephropathy commonly have diabetic retinopathy. The mechanisms by which chronic hyperglycemia leads to diabetic nephropathy, although poorly defined, involve the effects of factors like angiotensin II, renal microcirculation changes , and structural changes in the glomerulus[4]

The nephropathy associated with diabetes has been attributed to oxidative stress [8]. Oxidative stress can be caused either by the increased production of reactive oxygen species (ROS) or a deficiency in antioxidant defense. Antioxidant deficiency can result from low intake of vitamins, such as vitamin C and E, or impaired synthesis of enzymes, such as super oxide dismutase, catalase and glutathione peroxidase, due to zinc deficiency [6]. Chronic zinc deprivation generally results in an increased sensitivity to the effects of oxidative stress due to deficiency of these enzymes[7].


DIABETIC NEPHROPATHY
KIDNEY DISEASE
PROTEIN IN URINE
SECRETION OF PROTEINS
DILATED AFFERENT ARTERIOLE
UNHEALTHY GLOMERULUS
PROTEIN MOLECULES SPILL IN TO THE URINE BECAUSE OF DAMAGE OF CAPILLARY WALL
CONSTRICTED EFFERENT ARTERIOLE (HIGH PRESSURE)
KIDNEY
NEPHRONS
GLOMERULUS
URINE
NORMAL GLOMERULUS
CAPILLARY KEEPS PROTEIN MOLECULES IN THE BLOOD

HISTOPATHOLOGICAL CHANGES



The nephropathy that develops in type 2 DM differs from that of type 1 DM in the following respects: (1) microalbuminuria or macroalbuminuria may be present when type 2 DM is diagnosed, reflecting its long asymptomatic period; (2) hypertension more commonly accompanies microalbuminuria or macroalbuminuria in type 2 DM; and (3) microalbuminuria may be less predictive of diabetic nephropathy and likelihood of progression to macroalbuminuria in type 2 DM. albuminuria in type 2 DM may be secondary to factors unrelated to DM, such as hypertension, congestive heart failure (CHF), prostate disease, or infection.

Because some individuals with type 1 or type 2 DM have a decline in GFR in the absence of albuminuria, annual measurement of the serum creatinine to estimate GFR should also be performed. An annual

microalbuminuria measurement (albumin-to-creatinine ratio in spot urine) is advised in individuals with type 1 or type 2 DM

Screening for albuminuria should commence 5 years after the onset of type 1 DM and at the time of diagnosis of type 2 DM. Type IV renal tubular acidosis (hyporeninemic hypoaldosteronism) may occur in type 1 or 2 DM. These individuals develop a propensity for hyperkalemia and acidemia,.

DIABETIC NEUROPATHY

Diabetic neuropathy occurs in ~50% of individuals with long-standing type 1 and type 2 DM. Polyneuropathy, mononeuropathy and/or autonomic neuropathy are the different manifestations.The presence of CVD, elevated triglycerides, and hypertension is also associated with diabetic peripheral neuropathy.

The most common form of diabetic neuropathy is distal symmetric polyneuropathy[4]. Symptoms may include a sensation of numbness, tingling, sharpness, or burning that begins in the feet and spreads proximally. Pain typically involves the lower extremities, is usually present at rest, and worsens at night[4]. As diabetic neuropathy progresses, the pain subsides and eventually disappears, but a sensory deficit in the lower extremities persists. Physical examination reveals sensory loss, loss of ankle deep-tendon reflexes, and abnormal position sense.

Mononeuropathies most commonly invove the median, ulnar, and radial nerves. Mononeuropathy is less common than polyneuropathy in DM and presents with pain and motor weakness in the distribution of a single nerve.

Mononeuropathies can occur at entrapment sites such as carpal tunnel or be noncompressive.Mononeuropathy multiplex may also occur.

Involvement of the third cranial nerve is most common and is heralded by diplopia. Other cranial nerves, such as IV, VI, or VII may be affected.

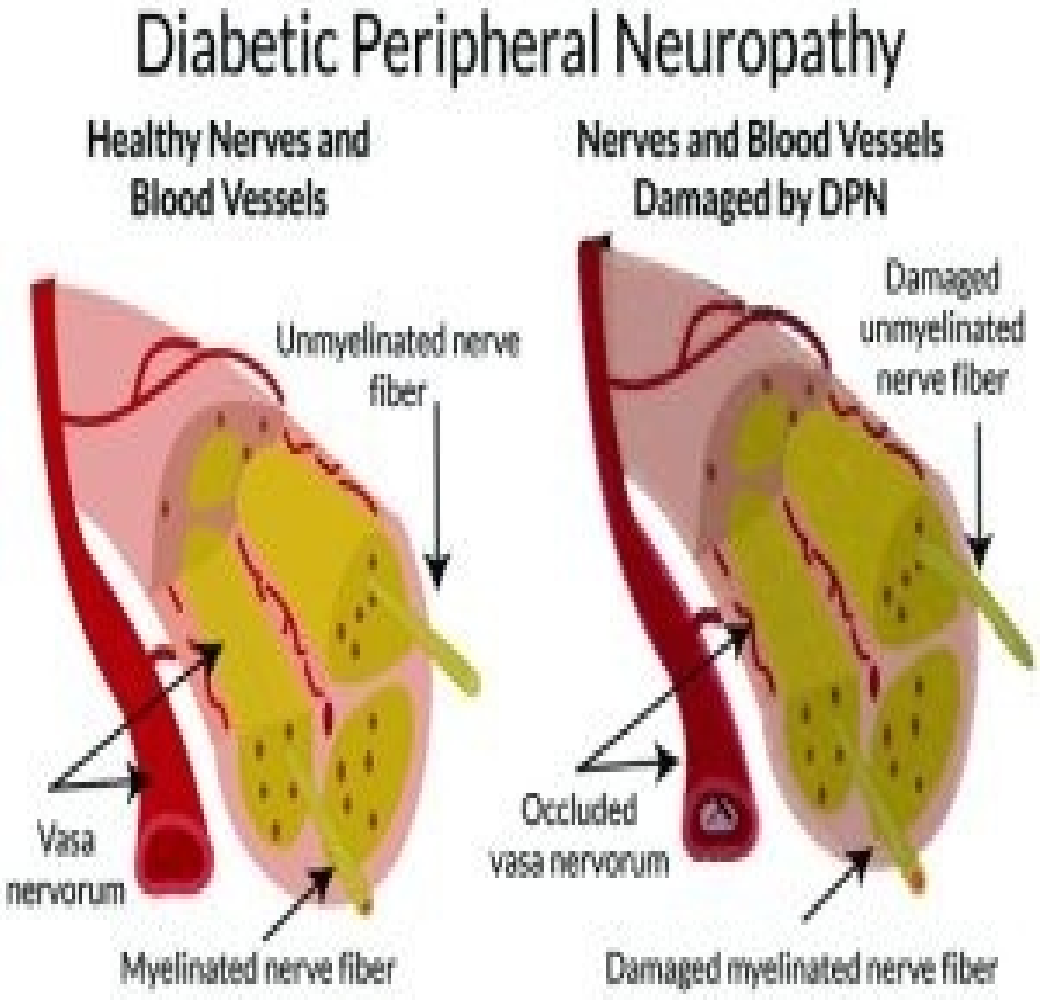
Diabetic polyradiculopathy is a syndrome characterized by severe disabling pain in the distribution of one or more nerve roots. It may be accompanied by motor weakness. Intercostal or truncal radiculopathy causes pain over the thorax or abdomen. Involvement of the lumbar plexus or femoral nerve may cause severe pain in the thigh or hip and may be associated with muscle weakness in the hip flexors or extensors (diabetic amyotrophy). Fortunately, diabetic polyradiculopathies are usually self-limited and resolve over 6–12 months.

Autonomic Neuropathy

Individuals with long-standing type 1 or 2 DM may develop signs of autonomic dysfunction involving the cholinergic, noradrenergic, and peptidergeric systems. DM-related autonomic neuropathy can involve multiple systems, including the cardiovascular, gastrointestinal, genitourinary, sudomotor, and metabolic systems.

Autonomic neuropathies affecting the cardiovascular system cause a resting tachycardia and orthostatic hypotension. Gastroparesis and bladder emptying abnormalities are often caused by the autonomic neuropathy seen in DM. Hyperhidrosis of the upper extremities and anhidrosis of the lower extremities result from sympathetic nervous

system dysfunction. Anhidrosis of the feet can promote dry skin with cracking, which increases the risk of foot ulcers.



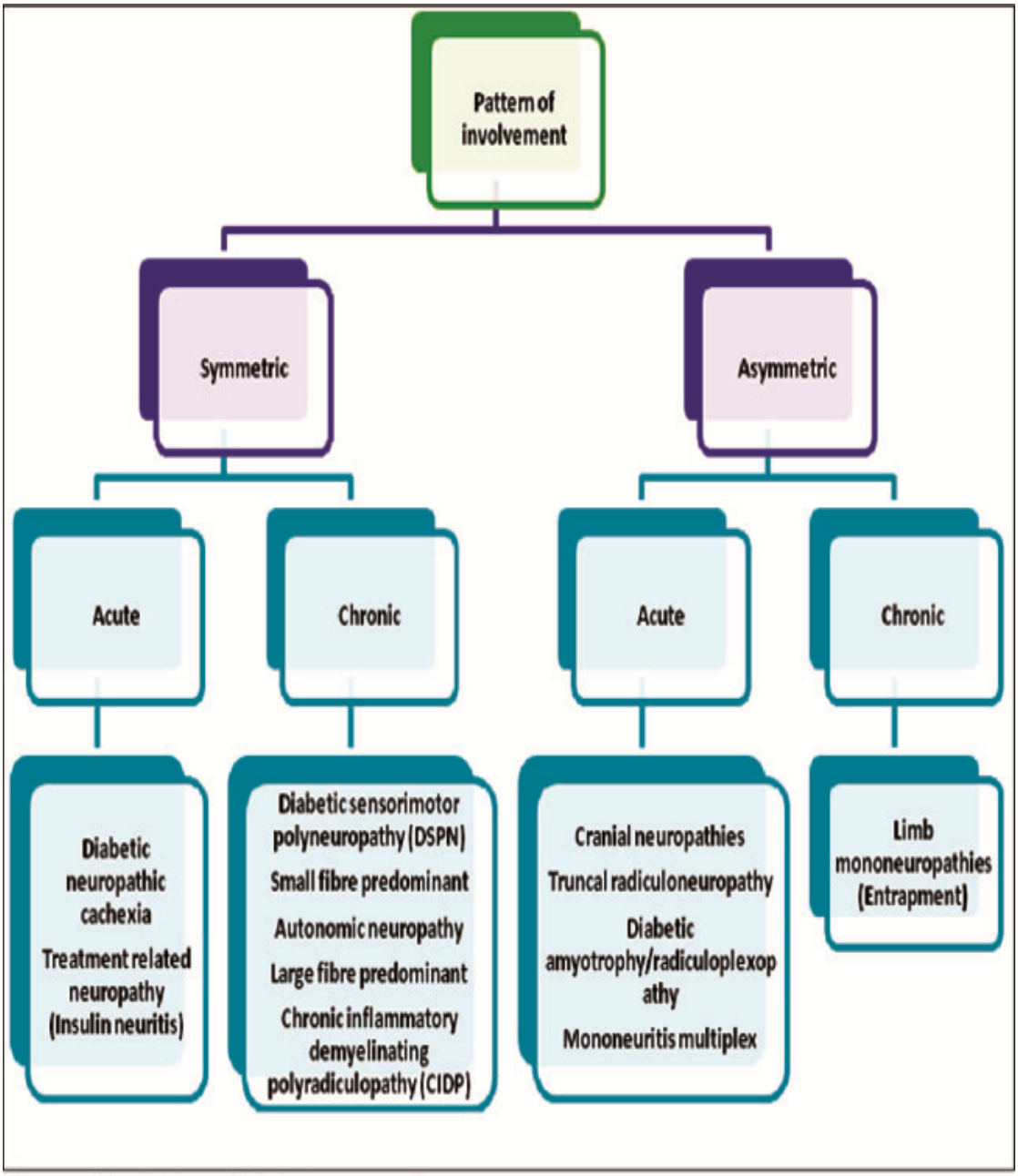
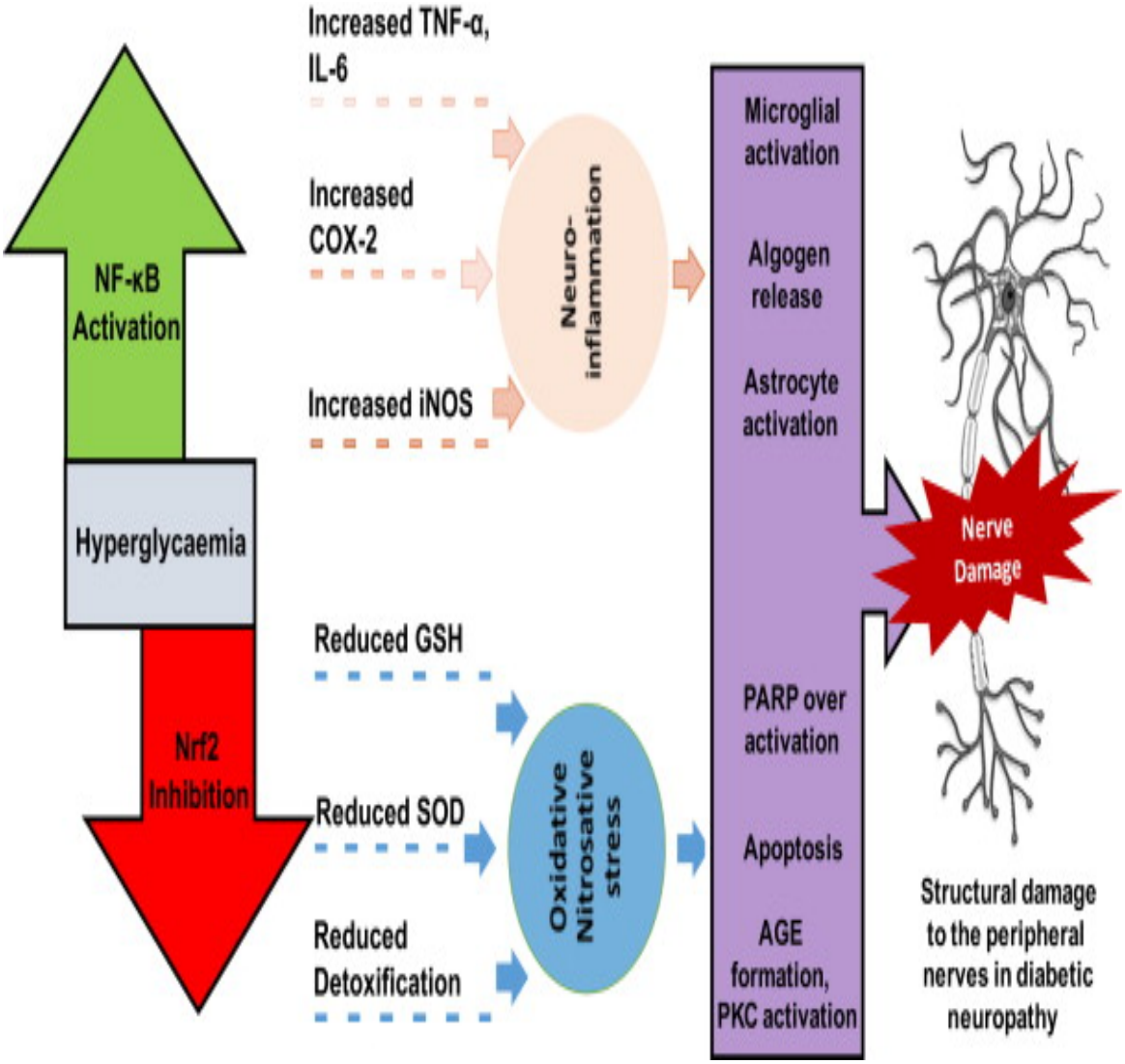
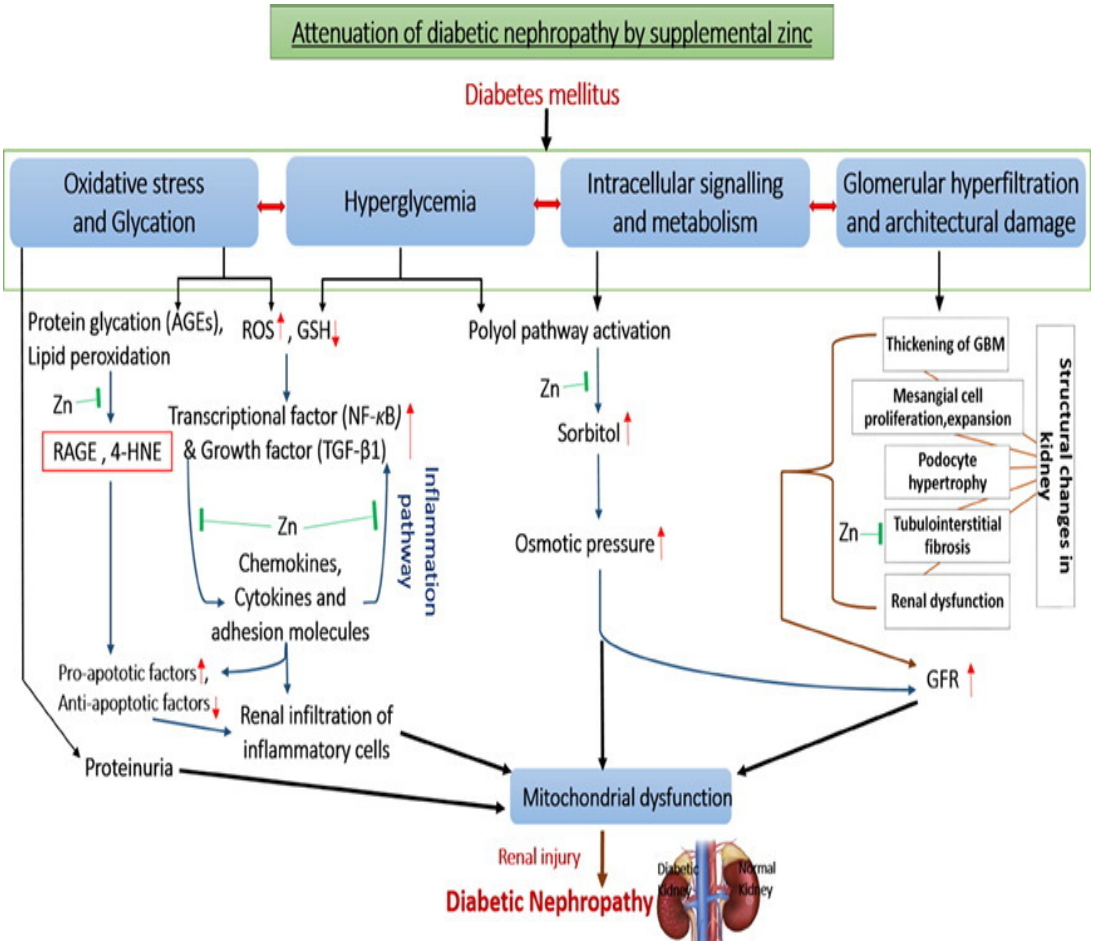


Figure: Classification of Diabetic neuropathy.



Zinc supplementation ameliorate severity of neuropathy symptoms in diabetic patients with mild to moderate peripheral neuropathy [24]. Zinc supplementations alone have also also demonstrated a significant improvement in motor nerve conduction velocity following supplementation in patients with type-2 diabetes [28].


Attenuation of diabetic nephropathy by supplemental zinc
Diabetes mellitus
Oxidative stress and Glycation
Hyperglycemia
Intracellular signalling and metabolism
Glomerular hyperfiltration and architectural damage
Protein glycation (AGEs), Lipid peroxidation
ROS, GSH
Polyol pathway activation
Zn
RAGE , 4-HNE
Transcriptional factor (NF-κB) & Growth factor (TGF-β1)
Inflammation pathway
Sorbitol
Osmotic pressure
Chemokines, Cytokines and adhesion molecules
Pro-apototic factors, Anti-apoptotic factors
Renal infiltration of inflammatory cells
Proteinuria
GFR
Thickening of GBM
Mesangial cell proliferation,expansion
Podocyte hypertrophy
Tubulointerstitial fibrosis
Renal dysfunction
Structural changes in kidney
Mitochondrial dysfunction
Renal injury
Diabetic Nephropathy
Diabetic kidney
Normal Kidney

1) Ying Ying Luo et al conducted a study in Peking University People's Hospital, Beijing, China, in 412 hospitalized patients with type 2 diabetes mellitus. The serum zinc levels between patients with specific microvascular complications and those without were compared. The association between zinc level and each microvascular complication. were analyzed. And concluded that lower serum zinc level in T2D patients lead to microvascular complications.Also hypozincemia is an independent risk factor for DN[60]. Older age, longer diabetes duration, higher HbA1c level, and the prevalence of DN were risk factors related to the lower serum zinc level[60].

2) Xiao Miao et al,Weixia Sun et al in a study conducted in The Second Hospital of Jilin University, China concluded that increased oxidative stress plays an important role in many human diseases, such as diabetes and its complication. Zn supplementation seems beneficial for the patients with diabetes to control glucose levels. Zn as an antioxidant or via induction of MT attenuates ROS effect[61]. Zn might protect retina from ROS induced pericytes apoptosis, capillary leakage, and neovascularization , thereby might have protective on DR[61].

3) Migdalis IN et al, Triantafilou P et al, conducted a study in NIMTS Hospital, Athens, Greece in seventy-seven patients with Type 2

diabetes (39 neuropathic and 38 non-neuropathic) and 38 control subjects and demonstrated a negative relationship between zinc level and lipid peroxidation and concluded diabetic neuropathy with low zinc level may be due to the elevated lipid peroxidation[63].

4): Dhia J., TimimiDhia et al did a study in the College of Medicine, University of Duhok, Iraq with 300 diabetic patients and 100 non-diabetic healthy subjects. The serum zinc levels in diabetic patients were compared to non-diabetic control subjects. Low- serum zinc was present in one third of patients with diabetic nephropathy, particularly among patients with microalbuminuria[64]. Low serum zinc levels leads to advancing nephropathy[64]**.**

5) Study of Zinc and Glycated Hb Levels in Diabetic Complications by B. Jyothirmayi et al, Department of Biochemistry, SRM Medical College Hospital & Research Centre, Kattankulathu showed that correlation of HbA1C and zinc was inversely related and diabetic complications were attributed to increased susceptability to lipid peroxidation due to free radical damage[68]. Strict glycemic control and zinc supplimentations can prevent complications to some extent.

MATERIALS AND METHOD

Study design : Cross-sectional comparative study

Study period : 6 months

Study area : Govt. Kilpauk Medical College ,Chennai.

Sampling : Simple random sampling

Study population:-

Diabetic patients attending the Medicine Department, Govt. Kilpauk Medical College, Chennai.

Sample Size

Sample size is calculated using the formula

$(1.96)^2$ pq/ d^2 Considering the prevalence a with CI 95% and power 80% *Sample size =120*

Inclusion criteria:-

Type 2 diabetic patients in the age group of 40-65 yrs

Exclusion criteria:-

Patients suffering from

- ✓ hypertension
- ✓ alcoholics, patients with Vit B12 deficiency
- ✓ with history of acute infections and thyroid dysfunction
- ✓ with auto immune diseases
- ✓ on chemotherapy
- ✓ non diabetic renal disease
- ✓ patients with macrovascular complications CAD,CVA,PVD

METHODOLOGY

After getting consent from patient or patiemt's relatives, following data will be collected from all diabetic patients.

Name, gender, age, duration of diabetes, treatment details including oral hypoglycemic drugs and insulin and detailed clinical examination will be done.

Diabetes was defined using the World Health Organization criteria:

(1) random plasma glucose ≥11.1 mmol/L(200mg/dl);

or

(2) fasting plasma glucose ≥7.0mmol/L (126 mg/dl);

or

(3) 2h glucose level in oral glucose tolerance test ≥11.1 mmol/L(200md/dl).

Fasting and Post Prandial blood sugars were done.

Zinc level was assayed by atomic absorption spectrophotometer.

MACHINE AND MODEL NO: Perkin Elmer 800AA.

REFERENCE RANGE 70-150 microgm/dl

Diabetic Retinopathy was diagnosed ophthalmologically by fundus examination.

Diabetic Nephropathy was by diagnosed by urinary protein/creatinine ratio. DN diagnosed if the urinary PCR was higher than.3. Urinary infection and other types of nephropathy were excluded during the diagnosis of DN.

Diabetic Neuropathy was diagnosed based on the results of physical examination and nerve conduction study.

- The data of each patient will be collected in specific proforma (ANNEXURE 2) which includes patient's name, age, sex, demographic details, presenting complaints, risk factors and all clinical data.

- All the relevant data and values are then entered in master chart in Microsoft excel format an then analyzed statiscially.

STATISTICAL ANALYSIS

The data was collected in the master chart obtained in the Microsoft excel format.

Descriptive statistics was done for all data and were reported in terms of mean values and percentages. Suitable statistical tests of comparison were done. Continuous variables were analysed with the unpaired t test and ANOVA. Categorical variables were analysed with the Chi-Square Test and Fisher Exact Test. Pearsons r correlation was done to assess relationship between variables. Statistical significance was taken as $P < 0.05$. The data was analysed using SPSS version 16 and Microsoft Excel 2007.

RESULTS

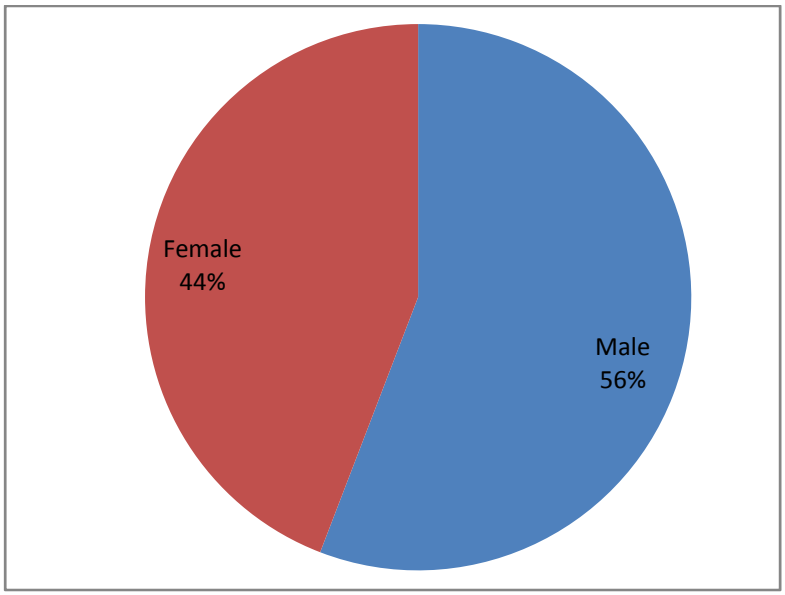
The total patients recruited in our study were 120. The following chart depict frequency distribution of gender.

GENDER:

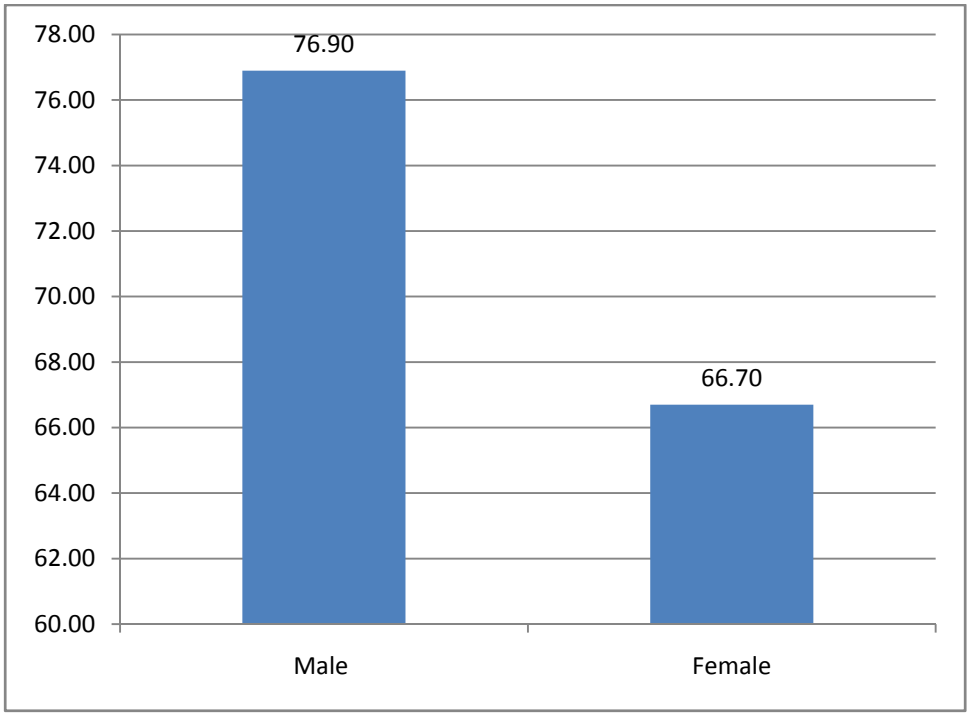
In a total of 120 patients participated in our study 67 patients were males and 53 patients were females. This distribution shows the predominance of males in type 2 diabetes mellitus.

GENDER	FREQUENCY	PERCENTAGE
MALE	67	55.8
FEMALE	53	44.2
TOTAL	120	100.0

Gender distribution



ASSOCIATION BETWEEN SERUM ZINC AND GENDER



Serum zinc levels when matched against gender status, it was observed that the mean serum zinc levels were 76.90±30.36 in male patients and 66.70±30.51 in female patients (p= 0.071). The data subjected to unpaired test reveals the existence of statistically non-significant association between serum zinc distribution and gender (p > 0.05).

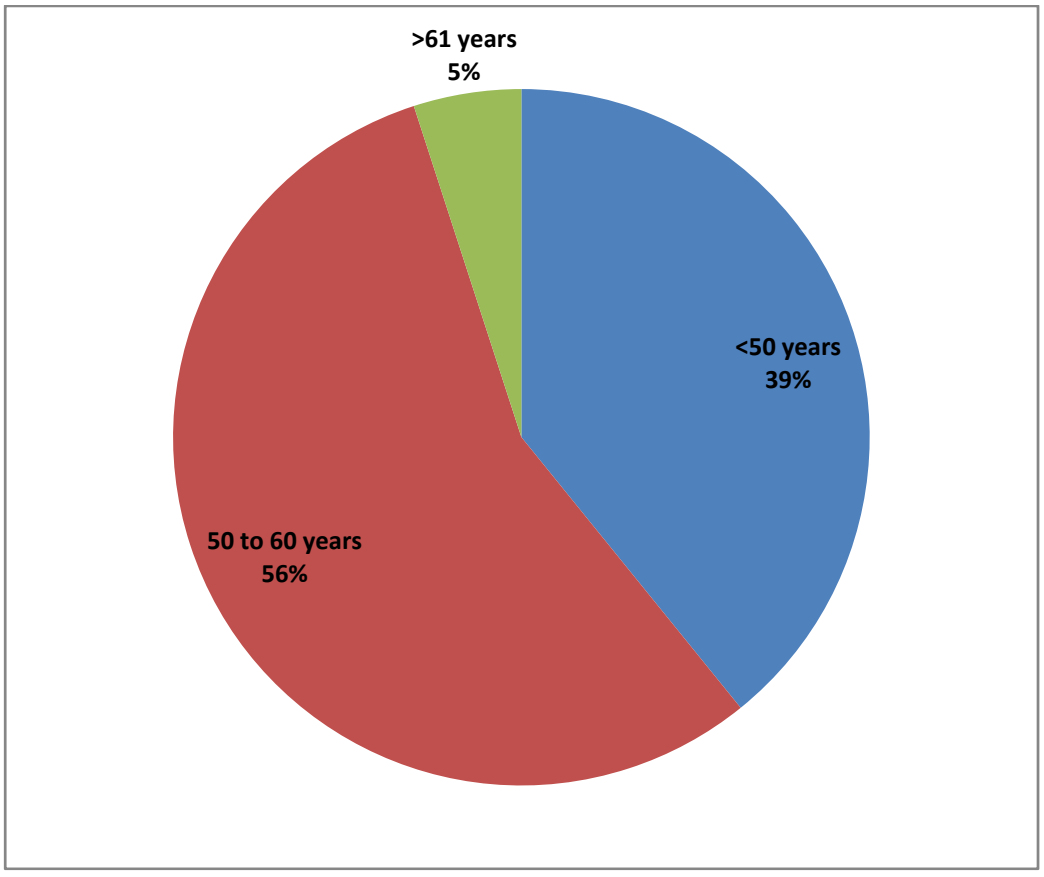
MEAN AND STANDARD DEVIATION OF VARIABLES:

	N	Mean	Std. Deviation
AGE	120	52.20	5.68
DM_DURATION	120	7.96	2.38
FBS	120	173.13	48.25
PPBS	120	218.07	55.43
ZINC	120	72.39	30.73
URINE PCR	120	0.52	0.89
VALID N (LISTWISE)	120		

Data collected from 120 selected T2DMsubjects were internally compared, tabulated, analysed and interpreted by using descriptive and inferential statistics based on the formulated objectives of the study. 56% of the study subjects were males. The mean age of the participants was 52.21 ± 5.68 years, and the mean duration of diabetes was 8 years. Mean fasting blood sugar levels tabulated was 173.13±48.25 and mean post prandial.

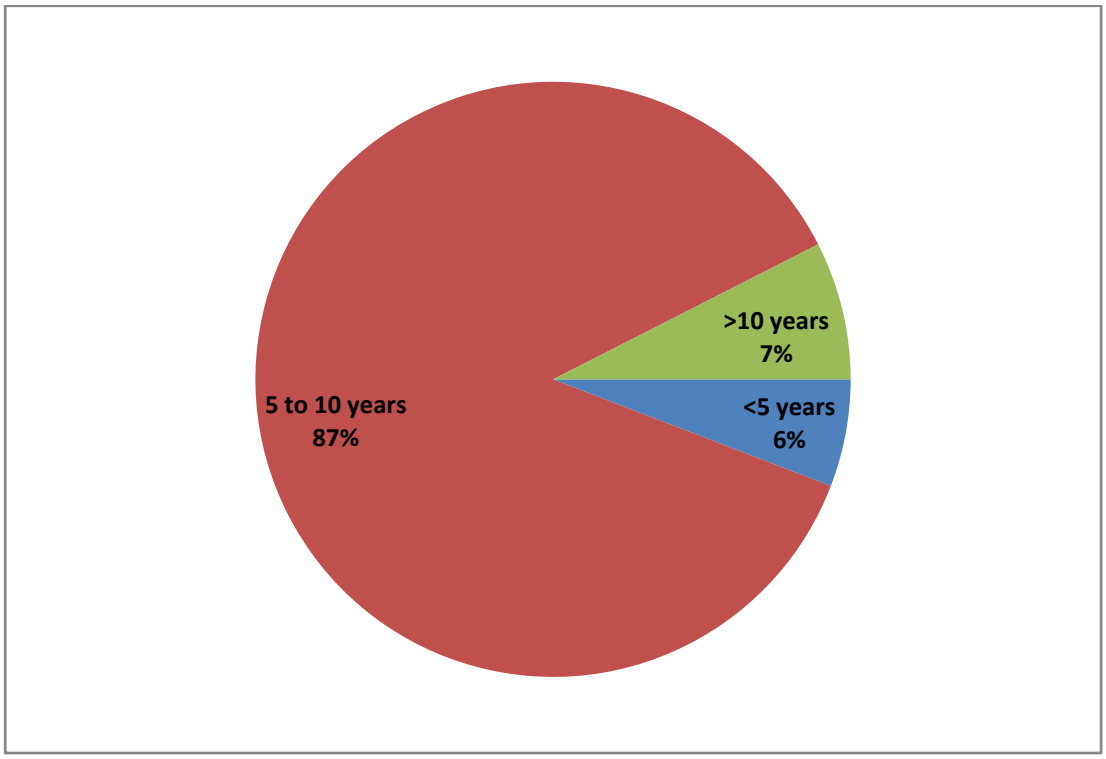
DISTRIBUTION OF AGE GROUP:

Age group	Frequency	Percent
<50 years	47	39.2%
50 to 60 years	67	55.8%
>61 years	6	5.0%



DISTRIBUTION OF DURATION OF DIABETES:

Duration of Diabetes	Frequency	Percent
<5 years	7	5.8%
5 to 10 years	104	86.7%
>10 years	9	7.5%



CORRELATION BETWEEN SERUM ZINC AND DURATION OF DIABETES

In patients with T2DM, when the serum zinc levels was matched and correlated with duration of diabetes, the mean serum zinc level was 72.39 μmol/L and the mean duration of diabetes was 7.96 years. The difference in values is statistically non-significant as the p value is 0.301with a negative correlation as per person's coefficient of -0.095. Blood sugar values were 218.07±55.43. Zinc levels exhibited values of 72.39±30.73 and urine PCR exhibited values were 0.52±0.89. 85% of the subjects had normal fundus and 11% had NPDR. 39% of the study population had impaired nerve conduction studies.

ASSOCIATION BETWEEN S.ZINC AND URINE PCR

Serum zinc levels when matched against urine PCR status, it was observed that the mean serum zinc levels were 81.63±27.34 in normal urine PCR category and 35.46±4.34 in elevated urine PCRcategory (p=<0.0001). The data subjected to unpaired t test reveals the existence of statistically significant association between serum zinc distribution and urine PCRstatus ($p < 0.05$).

CORRELATION BETWEEN SERUM ZINC AND URINE PCR

In patients with T2DM,when the serum zinc levels was matched and correlated with urine PCR levels, the mean urine zinc level was 72.39 μmol/L and the mean urine PCR level was 0.52 g/mmol. The difference in values is statistically significant as the p value is < 0.0001with a negative correlation as per pearson's coefficient of -0.479.

The decrease in serum zinc levels correlates negatively and strongly with the increase in urine PCR levels. The linear decrease in serum zinc level measurement in T2DM cases group in relation to increased urine PCR levels is true 48% of times.

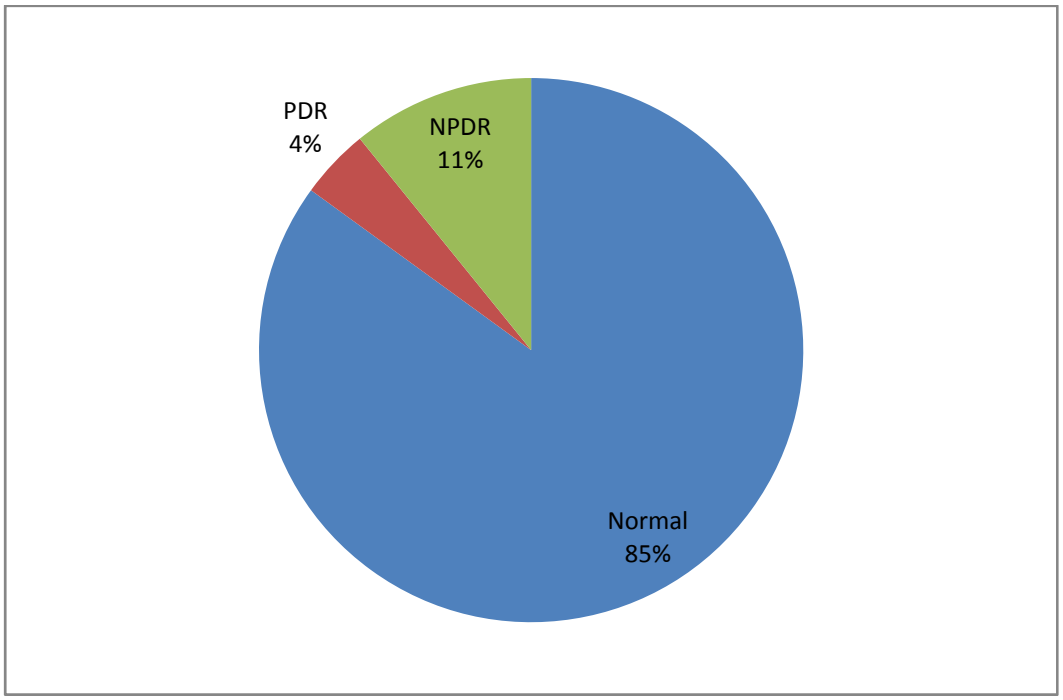
For every 1 % decrease in serum zinc levels there is a corresponding 0.22 % increase in urine PCR levels. This is indicated by the linear correlation formula $y = -0.0138x + 1.5169$.

DISTRIBUTION OF RETINOPATHY

DR	Frequency	Percent
Normal	102	85.0
PDR	5	4.2
NPDR	13	10.8
Total	120	100.0

Out of 120 patients in our study, 102 patients had normal fundus , 5 patients had PDR and 13 patients had NPDR.

DISTRIBUTION OF RETINOPATHY

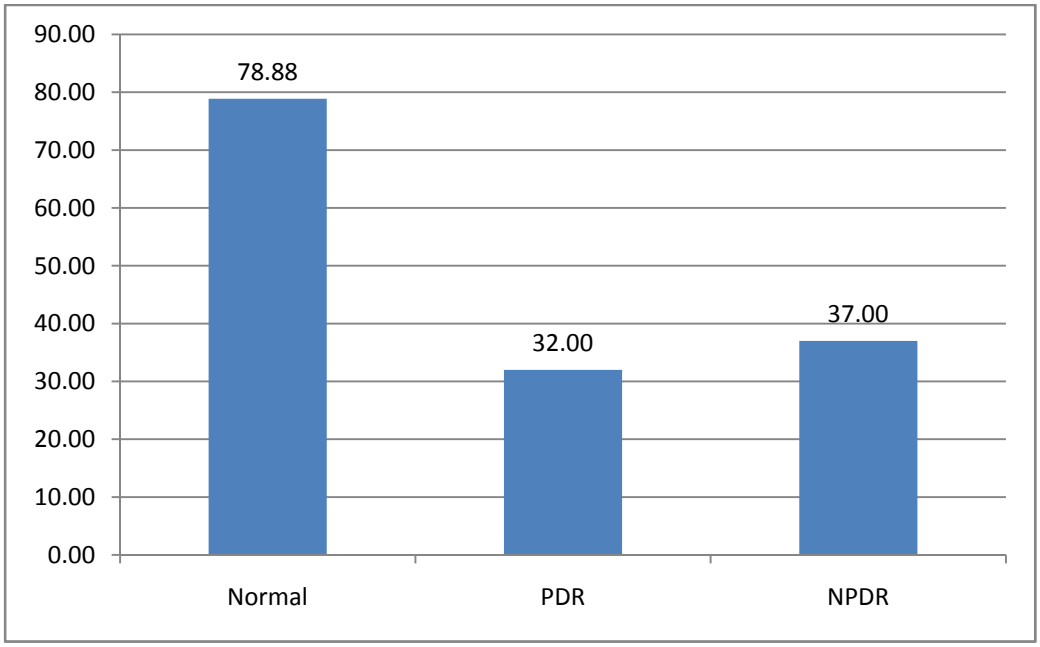


ASSOCIATION BETWEEN SERUM ZINC LEVELS AND DIABETIC RETINOPATHY

	N	Mean	Std. Deviation	P value
Normal	102	78.88	28.73	
PDR	5	32.00	2.00	
NPDR	13	37.00	4.22	<0.0001

Serum zinc levels when matched against retinopathy status, it was it was observed that the mean serum zinc levels were 78.88±28.73 in normal fundus category, 32.00±2.00 in proliferative diabetic retinopathy category and 37.00±4.22 in non-proliferative diabetic retinopathy category (p=<0.0001). The data subjected to ANOVA test reveals the existence of statistically significant association between serum zinc distribution and presence/severity of retinopathy ($p < 0.05$).

ASSOCIATION BETWEEN SERUM ZINC AND DIABETIC RETINOPATHY

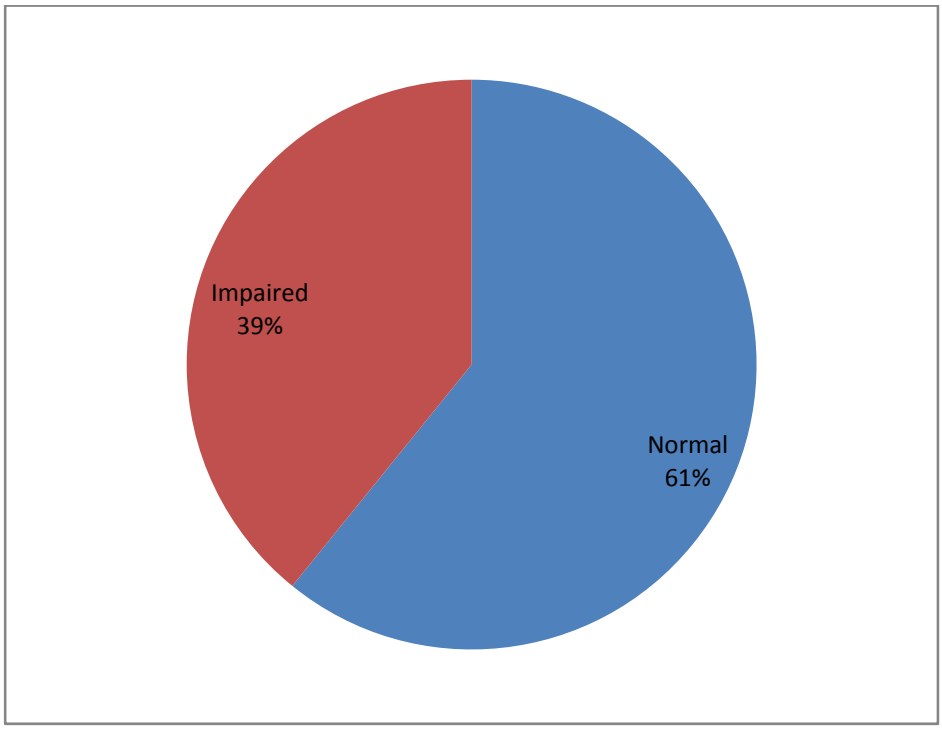


DISTRIBUTION OF DIABETIC PERIPHERAL NEUROPATHY

NCS	Frequency	Percent
Normal	73	60.8
Impaired	47	39.2
Total	120	100.0

In a total of 120 patients 73patients had normal NCS and 47 had impaired NCS

DISTRIBUTION OF PERIPHERALNEUROPATHY

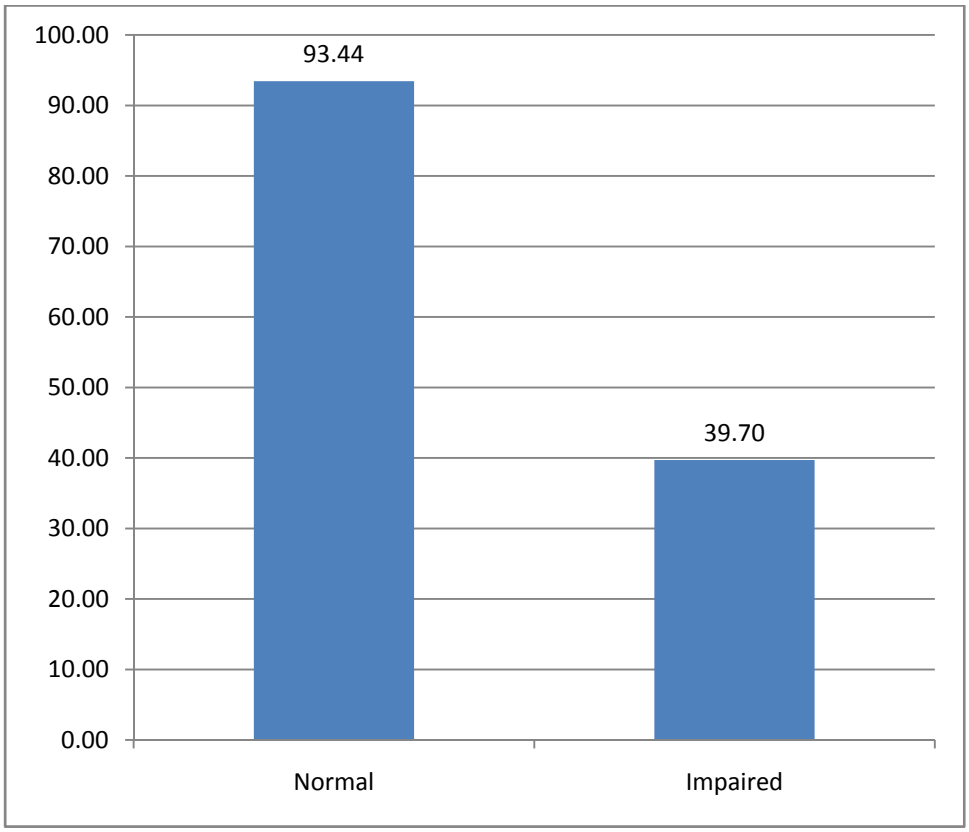


ASSOCIATION BETWEEN SERUM ZINC AND PERIPHERAL NEUROPATHY

NCS		N	Mean	Std. Deviation	P value
zinc	Normal	73	93.44	17.35	<0.0001
	Impaired	47	39.70	13.27	

Serum zinc levels when matched against nerve conduction study status, it was observed that the mean serum zinc levels were 93.44±17.35 in normal nerve conduction study patients, 39.70±13.27 in impaired nerve conduction study patients (p= <0.0001). The data subjected to chi squared test reveals the existence of statistically significant association between serum zinc distribution and nerve conduction study status ($p < 0.05$).

ASSOCIATION BETWEEN SERUM ZINC AND PERIPHERAL NEUROPATHY



DISCUSSION

In our study total of 120 patients were included. All patients were included in study after getting consent, detail history and physical examination and after ruling out the exclusion criteria. Out of 120 patients 67 patients were male and 53 patients were females. This distribution shows the predominance of males in type2 diabetes mellitus.. The mean age of the participants was 52.21 ± 5.68 years, and the mean duration of diabetes was 8 years.. Zinc levels exhibited values of 72.39±30.73 and urine PCR exhibited values were 0.52±0.89. 85% of the subjects had normal fundus and 11% had NPDR. 39% of the study population had impaired nerve conduction studies

In our study the distribution of mean serum zinc levels and the urine PCR status was meaningfully significant. This is evident by the decreased mean serum levels in elevated urine PCR category compared to normal urine PCR category (mean reduction difference of 46.14 percentage points, 57% lower).

The same view was echoed in a study conducted by Al Timimi DJ et al which showed that significantly low levels of e-GFR and high levels of microalbuminuria were observed in diabetic patients with low serum zinc level as compared to normal serum zinc level[59]. It concluded that

lower serum zinc levels leads to advancing nephropathy and indicated the need for determining serum zinc levels and the effectiveness of zinc supplementation in diabetic patients, particularly during the assessment of kidney damage[59]

Ying ying Lu et al in a study conducted in 412 patients concluded that zinc level was significantly lower in patients with elevated urinary albumin Creatinine ratio[60].And also suggested that serum zinc level was an independent risk factor for DN.

Barman S et al explored whether zinc supplementation protects against diabetic nephropathy through modulation of kidney oxidative stress and stress-induced expression related to the inflammatory process in streptozotocin-induced diabetic rats[61].This study concluded that zinc supplementation has a significant beneficial effect in the control of diabetic nephropathy. Which was exerted through a protective influence on oxidative-stress-induced cytokines, inflammatory proliferation and consequent renal injury[61].

On internal comparison the significant conclusion observed was that higher urine PCR status was related to decreased serum zinc levels (2.30 times more chance of developing elevated urine PCR). Lower zinc level is a good, consistent and direct predictor of high urine PCR or

diabetic nephropathy. Hence Lower serum zinc level can be considered as an independent risk factor for diabetic nephropathy.

In our study the distribution of mean serum zinc levels and the presence/severity of retinopathy status was meaningfully significant. This is evident by the decreased mean serum levels in non-proliferative diabetic retinopathy category compared to normal fundus category (mean reduction difference of 41.88percentage points, 53% lower) and decreased mean serum zinc levels in proliferative diabetic retinopathy category compared to non-proliferative diabetic retinopathy category (mean reduction difference of 5.00percentage points, 14% lower).

The same view was echoed in a study conducted in Peking University People's Hospital, Beijing, China, in 412 hospitalized patients with type 2 diabetes mellitus which concluded that lower zinc levels are found in DR patients than in those without DR , suggesting that zinc might play an important role in the development of DR[60]. Also suggested that in T2D patients with a relatively low zinc level, the protective effect of the anti-oxidative zinc may be reduced, and the risk of DR may be elevated[61].

A case control study conducted in 42 diabetic patients (14 without retinopathy [DC]; 14 with non-proliferative diabetic retinopathy [NPDR]; 14 with proliferative diabetic retinopathy [PDR]) at Ebin Al-Haitham

Specialized Hospital, Baghdad, Iraq showed significant reductions in serum means of Zn and Zn/Cu ratios in all diabetic retinopaths as compared to DC. And concluded that both glycation and oxidative processes were involved in the development of diabetic retinopathy, and changes in the concentration of Zn have some impact on the disease progression[62].

Miao X et al concluded that Zn supplementation seems beneficial for the patients with diabetes to control complications. Zn as an antioxidant or via induction of MT attenuates ROS effect. Zn might protect retina from ROS induced pericytes apoptosis, capillary leakage, and neovascularization thereby might have protective on DR[63].

On internal comparison the significant conclusion observed was that lower serum zinc level in Type 2 Diabetics patients was related to increased incidence of DR status (2.47 times more chance of developing diabetic retinopathy). Lower zinc level is a good,consistent and direct predictor of non-proliferative diabetic retinopathy and proliferative diabetic retinopathy. Hence lower serum zinc level can be considered as an independent risk factor for diabetic retinopathy

In our study the distribution of mean serum zinc levels and the nerve conduction study status was meaningfully significant. This is evident by the decreased mean serum levels in impaired nerve conduction

study category compared to normal nerve conduction study category (mean reduction difference of 53.74 percentage points, 58% lower).

The same view was echoed by a double-blind study conducted by Hayee et al which showed that serum zinc levels at baseline are significantly lower in patients with diabetic neuropathy when compared with healthy controls. conduction velocity were altered significantly in patients who received zinc supplement and conducted that zinc therapy may lead to better glycemic control and improvement in DPN[64].

Migdalis *et al* demonstrated a negative relationship between zinc level and lipid peroxidation[65]. Increased lipid peroxidation with low levels of zinc lead to neuropathy.

A double blind randomized study conducted on 50 subjects by Gupta R et al included 20 age and sex matched healthy controls ; 15 patients of diabetes mellitus with neuropathy received placebo for 6 weeks and 15 patients of diabetes mellitus with neuropathy were given supplemental zinc sulphate. It concluded that oral zinc supplementation helps in achieving better glycemic control and improvement in severity of peripheral neuropathy[66].

On internal comparison the significant conclusion observed was that decreased serum zinc level in Type 2 diabetics was related to

increased incidence of impaired nerve conduction study status (2.35 times more chance of developing diabetic neuropathy). Lower zinc level is a good, consistent and direct predictor of diabetic neuropathy. Hence Lower serum zinc level can be considered as an independent risk factor for diabetic neuropathy.

CONCLUSION

We can conclude that: Age, gender and glycaemic parameters and duration of diabetes had no statistically significant role to play on analysing the relationship between serum zinc level and microvascular complications in patients with type 2 diabetes.

On internal comparison the following conclusions were observed

- Lower zinc levels associated with abnormal linear increase in urine PCR levels
- Linear and inverse relationship with urine PCR
- Lower zinc levels associated with incidence of diabetic nephropathy
- Lower zinc levels associated with incidence of diabetic neuropathy and retinopathy

This study is a hypothesis proving study. Hence results have high clinical significance.

BIBLIOGRAPHY

1. Flannick J, Thorleifsson G, Beer NL, Jacobs SB, Grarup N, Burtt NP, *et al.* Loss‐of‐function mutations in SLC30A8 protect against type 2 diabetes. Nat Genet 2014;46:357‐63.

2. Shan Z, Bao W, Zhang Y, Rong Y, Wang X, Jin Y, *et al.* Interactions between zinc transporter‐8 gene (SLC30A8) and plasma zinc concentrations for impaired glucose regulation and type 2 diabetes. Diabetes 2014;63:1796‐803.

3. Thomas JP, Bachowski GJ, Girotti AW. Inhibition of cell membrane lipid peroxidation by cadmium‐ and zinc‐metallothioneins. Biochim Biophys Acta 1986;884:448‐61

4. Harrison's Principle of internal medicine 19 th edition

5. Diabetes Atlas - The Economic Impacts of Diabetes.

6. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes:estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27(5):1047–1053. doi: 10.2337/diacare.27.5.1047.

7. WHO Diabetes Fact Sheet.

8. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes:principles ofpathogenesis and therapy. Lancet. 2005;365(9467):1333–1346. doi: 10.1016/S0140-6736(05)61032-X.

9. American Diabetes Association: Diabetes Care 37(Suppl 1):S14,2014

10. Simon SF, Taylor CG. Dietary zinc supplementation attenuates hyperglycemia in db/db mice. Exp Biol Med(Maywood) 2001;226(1):43–51.

11. Faure P, Benhamou PY, Perard A, Halimi S, Roussel AM. Lipid peroxidation in insulin-dependent diabetic patients with early retina degenerative lesions: effects of an oral zinc supplementation. Eur J Clin Nutr. 1995; 49(4):282–288.

12. Shidfar F, Aghasi M, Vafa M, Heydari I, Hosseini S, Shidfar S.Effects of combination of zinc and vitamin A supplementation on serum fasting blood sugar,insulin, apoprotein B and apoprotein A-Iin patients with type i diabetes. Int J Food SciNutr. 2010;61(2):182–191. doi:10.3109/09637480903334171.

13. Afkhami-Ardekani M, Karimi M, Mohammadi SM, Nourani F.Effect of zinc sulfate supplementation on lipid and glucose in type 2 diabetic patients. PakJNutr.2008;7(4):550–553. doi:10.3923/pjn.2008.550.553.

14. Al-Maroof RA, Al-Sharbatti SS. Serum zinc levels in diabetic patients and effect of zinc supplementation on glycemic control of type 2 diabetics. Saudi Med J. 2006;27(3):344–350.

15. Anderson RA, Roussel AM, Zouari N, Mahjoub S, Matheau JM, Kerkeni A. Potential antioxidant effects of zinc and chromium supplementation in people with type 2 diabetes mellitus. J Am CollNutr. 2001;20(3):212–218.

16. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet. 2005;365(9467):1333–1346. -6736(05)61032-X.

17. Bădescu M, Păduraru I, Colev V, Saramet A, Bohotin C,Bădescu L. The relation zinc-lipidic peroxidation in experimentaldiabetes mellitus. Rom J Physiol. 1993;30(3–4):167–171.

18. Duzguner V, Kaya S. Effect of zinc on the lipid peroxidation and the antioxidant defense systems of the alloxan-induced diabetic rabbits. Free Radic Biol Med. 2007;42(10):1481–1486.

19. Tang Y, Yang Q, Lu J, Zhang X, Suen D, Tan Y, et al. Zinc supplementation partially prevents renal pathological changes in diabetic rats. J Nutr Biochem. 2010;21(3):237–46.

20. Wang X, Li H, Fan Z, Liu Y. Effect of zinc supplementation on type 2 diabetes parameters and liver metallothionein expressions inWistar rats. J Physiol Biochem. 2012;68(4):563–572.

21. Ohly P, Dohle C, Abel J, Seissler J, Gleichmann H. Zinc sulphate induces metallothionein in pancreatic islets of mice andprotects against diabetes induced by multiple low doses of streptozotocin. Diabetologia. 2000;43(8):1020–1030..

22. Özcelik D, Nazıroglu M, Tunçdemir M, Çelik Ö, Öztürk M,Flores-Arce MF. Zinc supplementation attenuates metallothionein and oxidative stress changes in kidney of streptozotocin-induced diabetic rats. Biol Trace Elem Res. 2012;150(1–3):342–349.

23. Zimny S, Gogolin F, Abel J, Gleichmann H. Metallothionein in isolated pancreatic islets of mice: induction by zinc and streptozotocin, a naturally occurring diabetogen. Arch Toxicol. 1993;67(1):61–65.

24. Wang J, Song Y, Elsherif L, Song Z, Zhou G, Prabhu SD, et al Cardiac metallothionein induction plays the major role in the prevention of diabetic cardiomyopathy by zinc supplementation. Circulation. 2006;113(4):544–54.

25. Zhang X, Liang D, Chi ZH, Chu Q, Zhao C, Ma RZ, et al. Effect of zinc on high glucose-induced epithelial-to-mesenchymal transition in renal tubular epithelial cells. Int J Mol Med.2015;35(6):1747–54]

26. Vijayaraghavan K, Iyyampillai S, Subramanian SP. Antioxidantpotential of zinc-flavonol complex studied in streptozotocindiabetic rats. J Diabetes. 2013;5(2):149–156. 0407.2012.00226.x

27. Moustafa SA. Zinc might protect oxidative changes in the retina and pancreas at the early stage of diabetic rats. Toxicol Appl Pharmacol. 2004;201(2):149–155.

28. Chu A, Foster M, Hancock D, Bell-Anderson K, Petocz P,Samman S. TNF-alpha gene expression is increased following zincsupplementation in type 2 diabetes mellitus. Genes &nutrition. 2015;10(1):440.

29. Liu F, Ma F, Kong G, Wu K, Deng Z, Wang H. Zinc supplementation alleviates diabetic peripheral neuropathy by inhibiting oxidative stress and upregulating metallothionein in peripheral nerves of diabetic rats. Biol Trace Elem Res. 2014;158(2):211–218.

30. Zhang X, Zhao Y, Chu Q, Wang ZY, Li H, Chi ZH. Zinc modulates high glucose-induced apoptosis by suppressing oxidative stress in renal tubular epithelial cells. Biol Trace Elem Res. 2014;158(2):259–267-

31. Garg VK, Gupta R, Goyal RK. Hypozincemia in diabetes mellitus. J Assoc Physicians India. 1994;42(9):720–721.

32. Pidduck HG, Wren PJ, Evans DA. Hyperzincuria of diabetes mellitus and possible genetical implications of this observation. Diabetes. 1970;19(4):240–247.

33. Jayawardena R, Ranasinghe P, Galappatthy P, Malkanthi R,Constantine G, Katulanda P. Effects of zinc supplementation ondiabetes mellitus: a systematic review and meta-analysis. DiabetolMetab Syndr. 2012;4(1):13. doi: 10.1186/1758-5996-4-13.

34. Liu F, Ma F, Kong G, Wu K, Deng Z, Wang H. Zincsupplementation alleviates diabetic peripheral neuropathy byinhibiting oxidative stress and upregulating metallothionein inperipheral nerves of diabetic rats. Biol Trace Ele Res. 2014;158(2):211–218. 9

35. Yoshikawa Y, Hirata R, Yasui H, Sakurai H. Alpha-glucosidase inhibitory effect of anti-diabetic metal ions and theircomplexes. Biochimie. 2009;91(10):1339–1341.

36. Zeng YF, Lee J, Si YX, Yan L, Kim TR, Qian GY, et al.Inhibitory effect of Zn2+ on α-glucosidase: Inhibition kinetics and molecular dynamics simulation. Process Biochem. 2012;47(12):2510–7.

37. Eckardt K, Schober A, Platzbecker B, Mracek T, Bing C,Trayhurn P, et al. The adipokine zinc-α2-glycoprotein activates AMP kinase in human primary skeletal muscle cells. Arch Physiol Biochem. 2011;117(2): 88–93.

38. Russell ST, Tisdale MJ. Studies on the anti-obesity activity ofzinc-α 2-glycoprotein in the rat. Int J Obes (Lond) 2011;35(5):658–665.

39. Miranda ER, Dey CS. Effect of chromium and zinc on insulin signaling in skeletal muscle cells. Biol Trace Elem Res. 2004;101(1):19–36. 10.1385/BTER:101:1:19.

40. Ceperuelo-Mallafre V, Ejarque M, Duran X, Pachon G,Vazquez-Carballo A, Roche K, et al. Zinc-alpha2-glycoprotein modulates AKT-dependent insulin signaling in human adipocytes by activation of the PP2A phosphatase. PLoS One. 2015;10(6):e0129644.

41. Buchner DA, Charrier A, Srinivasan E, Wang L, Paulsen MT, Ljungman M, et al. Zinc finger protein 407 (ZFP407) regulates insulin-stimulated glucose uptake and glucose transporter 4 (Glut4) mRNA. J Biol Chem. 2015;290(10):6376–86.

42. Tang X, Shay NF. Zinc has an insulin-like effect on glucose transport mediated by phosphoinositol-3-kinase and Akt in 3 T3-L1fibroblasts and adipocytes. J Nutr. 2001;131(5):1414–1420

43. Ilouz R, Kaidanovich O, Gurwitz D, Eldar-Finkelman H.Inhibition of glycogen synthase kinase-3beta by bivalent zinc ions:insight into the insulin-mimetic action of zinc. Biochem Biophys Res Commun. 2002;295(1):102–106. 291X(02)00636-8.

44. Ezaki O. IIb group metal ions (Zn2+, Cd2+, Hg2+) stimulate glucose transport activity by post-insulin receptor kinase mechanism in rat adipocytes. J Biol Chem. 1989;264(27):16118–16122.

45. Chimienti F, Devergnas S, Pattou F, Schult F, Garcia-Cuenca R, Vandewalle B, et al. In vivo expression and functional characterization of the zinc transporter ZnT8 in glucose-induced insulin secretion. J Cell Sci. 2006;119(20):4199–206.

46. Chimienti F, Devergnas S, Favier A, Seve M. Identification and cloning of a beta-cell-specific zinc transporter, ZnT-8, localized into insulin secretory granules. Diabetes. 2004;53(9):2330–2337.

47. Chimienti F, Favier A, Seve M. ZnT-8, a pancreatic beta-cellspecific zinc transporter. Biometals. 2005;18(4):313–317.

48. Wijesekara N, Dai FF, Hardy AB, Giglou PR, Bhattacharjee A,Koshkin V, et al. Beta cell-specific Znt8 deletion in mice causesmarked defects in insulin processing, crystallisation and secretion. Diabetologia. 2010;53(8):1656–68.

49. Nicolson TJ, Bellomo EA, Wijesekara N, Loder MK, BaldwinJM, Gyulkhandanyan AV, et al. Insulin storage and glucose homeostasis in mice null for the granule zinc transporter ZnT8 and studies of the type 2 diabetes-associated variants. Diabetes. 2009;58(9):2070

50. Shoae-Hagh P, Rahimifard M, Navaei-Nigjeh M, Baeeri M,Gholami M, Mohammadirad A, et al. Zinc oxide nanoparticles reduce apoptosis and oxidative stress values in isolated rat pancreatic islets. Biol Trace Elem Res. 2014;162(1–3):2629.

51. Gyulkhandanyan AV, Lu H, Lee SC, Bhattacharjee A,Wijesekara N, Fox JEM, et al. Investigation of transport mechanisms and regulation of intracellular Zn2+ in pancreatic alpha-cells. J Biol Chem. 2008;283(15):10184–97.

52. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;107(9):1058–1070.

53. Gerber PA, Bellomo EA, Hodson DJ, Meur G, Solomou A,Mitchell RK, et al. Hypoxia lowers SLC30A8/ZnT8 expression and free cytosolic Zn2+ in pancreatic beta cells. Diabetologia.2014;57(8):1635–44.

54. Pae EK, Kim G. Insulin production hampered by intermittent hypoxia via impaired zinc homeostasis. PLoS One. 2014;9(2):e90192.

55. Liu Y, Batchuluun B, Ho L, Zhu D, Prentice KJ, BhattacharjeeA, et al. Characterization of zinc influx transporters (ZIPS) in pancreatic beta cells: roles in regulating cytosolic zinc homeostasis and insulin secretion. J Biol Chem. 2015;290(30):18757–69

56. Hardy AB, Prentice KJ, Froese S, Liu Y, Andrews GK,Wheeler MB. Zip4 mediated zinc influx stimulates insulin secretion in pancreatic beta cells. PLoS One. 2015;10(3):e0119136.

57. Smidt K, Jessen N, Petersen AB, Larsen A, Magnusson N,Jeppesen JB, et al. SLC30A3 responds to glucose- and zinc variations in beta-cells

and is critical for insulin production and in vivo glucose-metabolism during beta-cell stress. PLoS One. 2009;4(5):e5684–4.

58. Myers SA, Nield A, Chew GS, Myers MA. The zinc transporter, Slc39a7 (Zip7) is implicated in glycaemic control in skeletal muscle cells. PLoS One. 2013;8(11):e79316.

59. Al-Timimi DJ, Sulieman DM, Hussen KR.J Clin Diagn Res. 2014 Nov;8(11):CC04-8

60. Luo YY, Zhao J, Han XY, Zhou XH, Wu J,Ji LN. Relationship Between Serum Zinc Level and Microvascular Complications in Patients with Type 2 Diabetes. Chin Med J

61. Barman S, Pradeep SR, Srinivasan K. J Nutr Biochem. 2018 Apr;54:113-129. doi: 10.1016/j.jnutbio.2017.11.008. Epub 2017 Dec 11.

62. Khan MI, Siddique KU, Ashfaq F, Ali W, Reddy HD, Mishra A. Effect of high-dose zinc supplementation with oral hypoglycemic agents on glycemic control and inflammation in type-2 diabetic nephropathy patients. J Nat Sci Biol Med. 2013;4:336–40.

63. Miao X, Sun W, Miao L, et al. Zinc and Diabetic Retinopathy. *Journal of Diabetes Research*. 2013;2013:425854. doi:10.1155/2013/425854.

64. Hayee MA, Mohammad QD, Haque A. Diabetic neuropathy and zinc therapy. Bangladesh Med Res Counc Bull. 2005;31:62–7.

65. Migdalis IN, Triantafilou P, Petridou E, Varvarigos N, Totolos V, Rigopoulos A. Lipid peroxides in type 2 diabetic patients with neuropathy. Res Commun Mol Pathol Pharmacol. 2005;117-118:5

66. Gupta R, Garg VK, Mathur DK, Goyal RK. Oral zinc therapy in diabetic neuropathy. J Assoc Physicians India.

PROFORMA

Name: Age/sex:

Address: OP NO:

DM – Yes/No

Diagnosis:

QUESTIONNAIRE:

Kuppusamy Socio economic status :

Duration of DM :

Type of treatment :

History of hypertension :

History of autoimmune diseases :

History of alcohol intake :

History of non diabetic kidney disease:

INVESTIGATIONS:

- FBS
- PPBS
- RFT
- LFT
- Hb
- Urine Protein
- Urine sediment
- Sr. zinc
- NCS
- Fundus examination.

சுயஒப்புதல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு:

இடம்: பொது மருத்துவத்துவ துரை
அரசு கீழ்பாக்கம் மருத்துவ கல்லூரி மருத்துவமனை
சென்னை

பங்குபெறுபவரின் பெயர் :

பங்குபெறுபவரின் வயது : **பங்குபெறுபவரின் எண் :**

மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்கள் எனக்கு விளக்கப்பட்டது. நான் இவ்வாய்வில் தன்னிச்சையாக பங்கேற்கிறேன். எந்த காரணத்தினாலோ எந்த சட்டசிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகிக்கொள்ளல்லாம் என்றும் அறிந்து கொண்டேன்.

இந்த ஆய்வு சம்பந்தமாகவோ,இதை சார்ந்து மேலும் ஆய்வு மேற்கொள்ளும்போதும் இந்த ஆய்வில்பங்கு பெறும் மருத்துவர் என்னுடைய மருத்துவ அறிக்கைகளை பார்ப்பதற்கு என் அனுமதி தேவையில்லை என அறிந்து கொள்கிறேன். இந்த ஆய்வின் மூலம் கிடைக்கும் தகவலையோ, முடிவையோ பயன்படுத்திக்கொள்ள மறுக்க மாட்டேன்.

இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக்கொள்கிறேன். இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்றும் உறுதியளிக்கிறேன்.

பங்கேற்பவரின் கையொப்பம் ஆய்வாளரின் கையொப்பம்

இடம் :

தேதி :

PATIENT CONSENT FORM

Study detail: **"Relationship Between Serum Zinc Level and Microvascular Complications in Patients with Type 2 Diabetes''**

Study centre : KILPAUK MEDICAL COLLEGE, CHENNAI
Patients Name :
Patients Age :
Identification Number :

Patient may check (✓) these boxes

I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask question and all my questions and doubts have been answered to my complete satisfaction. ☐

I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving reason, without my legal rights being affected. ☐

I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of current study and any further research that may be conducted in relation to it, even if I withdraw from the study I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study. ☐

I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperate with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or well-being or any unexpected or unusual symptoms. ☐

I hereby consent to participate in this study. ☐

I hereby give permission to undergo complete clinical examination and diagnostic tests including hematological, biochemical, radiological tests. ☐

Signature/thumb impression:

Patients Name and Address: place date

Signature of investigator :

Study investigator's Name : place date

s.no	age	sex	socioeconomic	op/ip	subsance use	education	marital status	family history	injury	duration	side of brain affected	lobe affected	GCS	positive	negative	general psychopathology	HAM D	HAM A	DSM V(personality)	ICD 10
1	33	m	l	ip	use	nil	married	nil	RTA	1	bilateral	f,t,o	2	7	7	22	4	4		
2	30	m	l	op	nil	nil	married	nil	RTA	4	left	t,p	2	14	10	24	5	5		
3	29	m	l	ip	nil	nil	married	nil	RTA	1	right	f	2	8	7	20	2	2	yes	
4	45	f	l	ip	nil	primary	married	nil	fall	1	left	f,t,p	2	10	7	17	5	16		
5	57	m	l	ip	use	nil	married	nil	RTA	1	left	f,t,p	2	7	8	25	4	6		
6	19	m	l	ip	nil	primary	unmarried	nil	RTA	1	right	t,p	2	8	7	18	5	7		
7	32	m	l	ip	nil	nil	married	nil	RTA	1	left	f,t,p	2	9	7	20	6	3		
8	45	m	l	ip	nil	nil	married	nil	RTA	1	left	t	2	12	9	21	7	2		
9	45	m	l	ip	nil	primary	married	nil	RTA	1	right	t	1	13	7	16	6	4		
10	35	m	l	ip	nil	nil	married	nil	RTA	1	right	p	1	9	7	18	5	3		
11	38	m	l	ip	nil	degree	married	nil	RTA	1	right	p	1	8	7	19	8	4		
12	40	m	l	ip	nil	nil	married	nil	RTA	1	right	t	2	10	8	20	4	11		
13	42	m	l	ip	nil	nil	married	nil	RTA	4	left	f,t,p	2	8	7	19	2	3		
14	30	m	l	ip	nil	degree	married	nil	TTA	3	right	f,t,p	3	12	9	22	3	6		organic catatonia
15	36	m	l	ip	use	nil	married	nil	assault	1	left	f,t	1	10	7	23	1	8	yes	
16	75	f	l	ip	nil	primary	married	nil	RTA	1	bilateral	f	2	7	9	27	16	2		
17	45	f	l	ip	nil	nil	married	nil	RTA	1	left	f, BG	2	8	8	26	17	4		
18	47	m	l	ip	nil	nil	married	nil	RTA	1	right	f	2	9	7	21	9	12		
19	44	f	l	ip	nil	primary	married	nil	RTA	1	left	t	1	19	12	20	5	6		
20	41	m	l	ip	use	primary	married	nil	RTA	1	right	f	1	11	9	18	3	8		
21	65	m	l	ip	nil	primary	married	nil	fall	1	bilateral	f,t,p	2	9	8	25	20	15		
22	37	m	l	ip	nil	primary	married	nil	RTA	1	right	t	1	8	9	18	4	8		
23	38	m	l	op	nil	nil	married	nil	RTA	3	left	f,p	1	12	10	19	4	7	yes	
24	33	m	l	ip	nil	nil	married	nil	RTA	1	left	p	1	7	11	20	5	7		
25	38	m	l	ip	nil	degree	married	nil	RTA	1	left	f,t	2	9	8	17	2	6		
26	32	m	l	ip	nil	nil	married	nil	RTA	1	left	f,p	1	7	9	18	5	8		
27	30	m	l	ip	nil	primary	married	nil	RTA	1	bilateral	f,p	2	8	10	20	12	5		
28	29	m	l	ip	nil	primary	married	nil	RTA	1	left	t,p	1	11	12	21	4	9		
29	43	m	l	ip	nil	nil	married	yes	RTA	1	left	t,p	3	22	9	19	3	12		
30	38	m	l	ip	use	primary	married	nil	RTA	1	left	f	2	10	10	19	4	6		
31	46	f	l	ip	nil	primary	married	nil	RTA	1	bilateral	f,t	2	8	7	22	8	4		
32	49	f	l	ip	nil	nil	married	nil	RTA	1	left	f,t	1	7	9	20	6	9		
33	45	f	l	ip	nil	nil	married	nil	RTA	2	right	o	2	10	8	19	3	17		
34	37	f	l	ip	nil	primary	married	nil	RTA	1	left	f,p	2	11	13	17	4	7		
35	42	f	l	ip	nil	primary	married	nil	RTA	1	bilateral	o	1	8	11	19	5	14		
36	34	m	l	ip	nil	primary	married	nil	RTA	1	right	t,o	2	16	7	21	2	3		
37	47	m	l	ip	nil	primary	married	nil	RTA	1	right	f	2	9	9	18	6	8		
38	22	m	l	ip	use	nil	unmarried	nil	RTA	2	bilateral	f	2	14	8	19	5	11	yes	
39	48	f	l	ip	nil	nil	married	nil	RTA	1	right	f,p	2	11	7	20	7	10		
40	39	m	l	ip	use	primary	married	nil	RTA	1	left	t	2	10	11	21	9	8		
41	20	m	l	ip	use	nil	unmarried	nil	RTA	1	right	f	1	8	10	19	3	9		
42	35	f	l	ip	nil	primary	married	nil	RTA	2	right	t,p	2	9	12	17	5	16		
43	47	m	l	ip	nil	nil	married	nil	RTA	1	right	f,t	1	7	7	21	7	8		
44	35	m	l	ip	use	nil	married	nil	RTA	1	left	f,BG	2	7	8	18	14	8		
45	42	f	l	ip	nil	primary	married	nil	RTA	1	left	p	1	7	19	17	6	9		
46	42	f	l	ip	nil	nil	married	nil	RTA	2	left	p	2	8	10	19	4	16		
47	33	m	l	ip	nil	nil	married	nil	RTA	1	right	f	1	10	7	21	5	7		
48	37	m	l	ip	nil	nil	married	nil	RTA	1	right	p	1	7	7	20	4	5		
49	66	m	l	ip	use	nil	married	yes	fall	1	left	f,p	3	8	14	28	21	16		
50	61	m	l	ip	nil	nil	married	nil	fall	1	right	f	2	7	9	19	5	4		
51	36	m	l	ip	nil	degree	married	nil	RTA	1	right	t	2	10	15	18	5	5		
51	47	m	l	ip	use	nil	married	nil	RTA	1	left	f	2	7	8	20	4	3		
53	58	m	l	ip	nil	nil	married	nil	RTA	1	left	t	2	8	9	21	5	5		
54	38	f	l	ip	nil	primary	married	nil	RTA	1	left	f	2	10	8	27	17	7		
55	51	f	l	ip	nil	nil	married	nil	RTA	1	right	o	1	7	7	19	6	4		
56	58	f	l	ip	nil	nil	married	nil	RTA	1	right	f,p	2	8	9	18	4	14		
57	57	m	l	ip	use	nil	married	nil	RTA	1	left	f,p	2	9	7	17	8	9		
58	33	m	l	ip	nil	primary	married	nil	RTA	1	right	t,o	2	7	9	22	7	10		

59	38	m	l	ip	nil	primary	married	nil	RTA	1	right	t,p	1	7	7	21	5	6		
60	44	f	l	ip	nil	primary	married	nil	RTA	1	bilateral	f,p	1	7	11	20	9	8		
61	43	m	l	ip	use	primary	unmarried	nil	RTA	3	bilateral	f	2	12	9	18	6	9	yes	
62	49	m	l	ip	nil	primary	married	nil	RTA	1	left	p	2	9	8	17	7	5		
63	58	m	l	ip	nil	primary	married	nil	RTA	1	bilateral	f,t,p	3	8	12	25	19	17		
64	52	f	l	ip	nil	primary	married	nil	RTA	1	right	f	1	10	9	17	6	4		
65	61	m	l	ip	nil	nil	married	nil	fall	1	left	f	2	8	7	16	5	7		
66	58	m	l	ip	nil	nil	married	nil	RTA	1	right	f,p	2	10	14	18	12	4		
67	55	f	l	ip	nil	nil	married	nil	RTA	1	right	t,p	1	8	9	17	4	6		
68	21	m	l	ip	use	primary	unmarried	nil	RTA	1	left	f	2	8	8	21	5	3		
69	36	m	l	ip	nil	primary	married	nil	RTA	2	right	f	2	9	9	22	3	15		
70	44	m	l	ip	nil	nil	married	nil	RTA	1	left	t,p	2	7	8	16	2	5		
71	28	m	l	ip	use	primary	married	nil	assault	3	right	f	1	13	10	18	6	8	yes	
72	39	f	l	ip	nil	primary	married	nil	RTA	1	left	p	2	10	7	17	14	6		
73	34	m	l	ip	nil	primary	married	nil	RTA	2	left	f	2	8	9	16	4	16		
74	67	m	l	ip	nil	primary	married	nil	RTA	1	left	f,t	2	7	8	22	3	12		
75	33	f	l	ip	nil	primary	married	nil	RTA	1	left	f	2	7	20	21	9	4		
76	49	m	l	ip	nil	nil	married	nil	RTA	1	right	f,p	1	8	7	16	4	10		
77	35	m	l	ip	nil	nil	married	nil	RTA	1	bilateral	f	1	10	9	17	8	5		
78	29	f	l	ip	nil	degree	unmarried	nil	RTA	1	right	f	1	9	11	16	3	3		
79	34	m	l	op	use	nil	married	nil	RTA	3	left	f,p	2	7	10	19	11	5		
80	42	m	l	ip	nil	primary	married	nil	RTA	1	right	f,t,p	3	9	8	18	6	17		
81	39	m	l	ip	nil	primary	married	nil	RTA	1	left	f,t	1	8	9	16	4	7		
82	72	f	l	ip	nil	primary	married	nil	fall	1	right	f,p	2	7	7	18	10	4		
83	44	f	l	ip	nil	primary	married	nil	RTA	1	right	f	1	20	11	17	2	5		
84	57	m	l	ip	use	nil	married	nil	RTA	3	right	f	2	7	9	16	5	8		
85	32	m	l	op	nil	nil	married	nil	RTA	3	left	f	2	8	9	17	4	6		
86	46	f	l	ip	nil	nil	married	nil	RTA	1	left	f,t	2	8	7	19	7	9		
87	44	m	l	ip	nil	nil	married	nil	RTA	1	left	f	2	10	7	20	5	6		
88	57	m	l	ip	nil	nil	married	nil	RTA	1	bilteral	f,t,p	3	8	10	18	17	7		
89	42	f	l	ip	nil	nil	married	nil	RTA	1	left	f	2	7	8	16	12	5		
90	41	m	l	ip	nil	nil	married	nil	RTA	1	right	f	1	7	9	17	3	8		
91	36	m	l	ip	nil	nil	married	nil	RTA	3	left	t,o	2	7	13	19	13	5		
92	34	f	l	ip	nil	nil	married	nil	RTA	3	left	f,t	1	7	24	18	2	12		
93	33	m	l	ip	nil	nil	married	nil	RTA	1	left	f	2	8	9	17	10	4		
94	28	m	l	ip	use	nil	unmarried	nil	RTA	1	right	f	1	7	12	20	5	7		
95	65	f	l	ip	nil	nil	married	nil	RTA	2	left	f,p	2	7	9	23	14	15		
96	33	m	l	ip	use	degree	married	nil	RTA	1	right	f,t	1	7	11	16	6	6		
97	39	m	l	ip	nil	primary	married	nil	RTA	1	right	f	1	7	10	18	9	5		
98	31	m	l	ip	use	nil	married	nil	RTA	1	right	f	1	7	9	17	4	12		
99	38	f	l	ip	nil	degree	married	nil	RTA	1	left	f	2	8	8	22	15	6		
100	27	m	l	ip	nil	primary	unmarried	yes	RTA	1	left	t	1	7	9	16	5	3		

S.NO.	AGE	SEX		Duration of DM (in yrs)	FBS (mg/dl)	PPBS (mg/dl)	SERUM ZINC (µg/dl)	URINE PCR	fundus examination	NCS
1	52	F		8	202	232	86	0.12	Normal	normal
2	42	F		3	186	211	90	0.2	Normal	normal
3	40	M		3.5	103	196	100	0.08	Normal	normal
4	61	M		10	170	191	88	0.15	Normal	normal
5	48	F		6	154	189	95	0.26	Normal	normal
6	47	M		7	132	186	102	0.13	Normal	normal
7	58	F		10	130	192	87	0.14	normal	normal
8	65	M		7	240	300	106	0.22	normal	normal
9	58	M		5	212	245	110	0.28	normal	normal
10	60	M		15	179	312	83	0.2	normal	normal
11	45	F		2	110	140	115	0.13	normal	normal
12	55	F		8	211	203	36	0.26	normal	impaired
13	53	F		8	106	210	40	0.12	normal	impaired
14	51	M		10	201	237	98	0.16	normal	normal
15	47	M		7	146	150	96	0.17	normal	normal
16	54	M		5	192	200	32	0.4	normal	impaired
17	53	F		12	241	323	40	0.45	normal	impaired
18	55	f		8	106	163	100	0.12	normal	normal
19	58	f		10	211	262	38	0.6	npdr	impaired
20	60	f		6	213	182	41	1.2	npdr	normal
21	48	f		7	104	145	118	0.13	normal	normal
22	53	M		8	96	133	107	0.2	normal	normal
23	50	M		10	85	140	118	0.16	normal	normal
24	54	M		8	162	183	118	0.22	Normal	normal
25	50	M		10	176	209	104	0.12	Normal	normal
26	49	M		7	132	201	98	0.22	normal	normal
27	51	M		10	201	182	86	0.28	normal	normal
28	55	M		4	180	173	101	0.16	normal	normal
29	48	M		8	200	201	98	0.24	normal	normal
30	60	M		12	211	187	103	0.23	normal	normal
31	50	M		9	243	212	94	0.16	normal	normal
32	58	M		6	132	163	106	0.26	normal	normal
33	52	M		7	165	180	90	0.14	normal	normal
34	51	M		9	132	174	89	0.16	normal	normal
35	42	F		6	183	265	35	0.13	normal	normal
36	55	M		10	160	270	30	1.2	NPDR	impaired
37	53	M		9	133	173	101	0.13	normal	impaired
38	45	M		9	160	203	43	0.12	normal	impaired
39	60	M		9	145	226	38	0.22	normal	impaired
40	54	M		7	256	280	32	1.8	npdr	impaired
41	45	M		10	133	160	40	0.2	normal	impaired
42	56	M		8	145	213	36	0.1	normal	impaired
43	45	M		6	256	246	42	0.26	normal	impaired
44	60	F		9	133	201	45	0.22	normal	impaired
45	50	M		6	201	173	35	0.1	normal	impaired
46	59	F		8	146	256	38	3	npdr	impaired
47	56	F		12	180	353	40	2.3	npdr	impaired
48	50	M		10	153	193	88	0.12	normal	normal
49	50	F		11	270	380	30	4	pdr	impaired
50	41	M		7	133	180	88	0.12	normal	normal
51	59	M		11	260	342	34	4	pdr	impaired
52	46	M		6	143	176	102	0.24	normal	normal
53	50	M		8	243	301	41	3	npdr	impaired

54	52	F		7	132	182	98	0.12	normal	normal
55	53	M		4	179	201	89	0.16	normal	normal
56	42	F		15	121	153	114	0.22	normal	normal
57	60	M		12	132	182	101	0.26	normal	normal
58	59	F		9	180	233	98	0.12	normal	normal
59	51	F		7	123	176	106	0.13	normal	normal
60	46	F		12	198	289	30	0.6	pdr	impaired
61	60	F		12	154	201	97	0.22	normal	normal
62	46	F		6	169	198	88	0.1	normal	normal
63	45	M		7	143	178	82	0.21	normal	normal
64	63	F		10	201	263	42	2.1	npdr	normal
65	52	F		8	132	201	110	0.12	normal	normal
66	58	M		9	142	232	39	0.26	normal	impaired
67	60	M		10	201	288	30	0.4	npdr	impaired
68	50	M		8	232	268	34	3	pdr	impaired
69	42	F		9	243	152	102	0.1	normal	normal
70	51	F		10	143	267	34	1.2	npdr	impaired
71	44	M		11	164	245	36	0.6	npdr	normal
72	49	M		8	110	197	108	0.12	normal	normal
73	54	F		14	265	301	40	3	npdr	normal
74	59	M		10	157	200	114	0.17	normal	normal
75	40	M		5	132	187	98	0.12	normal	normal
76	54	M		8	156	188	96	0.12	normal	impaired
77	52	M		7	233	276	39	0.22	normal	impaired
78	47	M		9	176	201	101	0.12	normal	normal
79	49	F		9	132	182	88	0.21	normal	normal
80	60	F		11	153	190	40	0.16	normal	impaired
81	55	F		5	156	190	37	0.27	normal	impaired
82	60	M		10	160	207	35	0.18	normal	impaired
83	56	F		7	154	198	88	0.16	normal	normal
84	52	F		9	132	176	85	0.12	normal	normal
85	41	F		6	214	300	34	0.23	normal	impaired
86	52	F		9	201	231	96	0.11	normal	normal
87	57	F		10	126	170	89	0.24	normal	normal
88	55	F		8	187	259	45	0.23	normal	impaired
89	65	M		5	282	332	30	0.21	normal	impaired
90	60	F		10	201	233	42	0.21	normal	impaired
91	58	F		7	133	183	44	0.1	normal	impaired
92	45	F		6	179	201	40	0.22	normal	impaired
93	49	F		8	183	267	38	0.13	normal	impaired
94	50	F		4	400	450	30	3	normal	impaired
95	55	F		6	298	350	32	3.5	pdr	impaired
96	53	F		5	259	365	39	0.2	npdr	impaired
97	58	M		6	199	280	42	0.22	normal	impaired
98	48	F		8	163	183	37	0.13	normal	impaired
99	42	M		9	170	221	41	0.16	normal	impaired
100	49	M		8	156	189	88	0.12	Normal	normal
101	48	F		7	133	180	98	0.09	normal	normal
102	52	M		6	143	187	100	0.12	normal	normal
103	51	M		7	176	201	88	0.16	normal	normal
104	47	M		6	187	234	98	0.23	normal	normal
105	55	M		9	145	187	90	0.2	normal	normal
106	53	M		6	176	204	96	0.15	normal	normal
107	54	M		9	167	189	101	0.1	normal	normal
108	58	M		7	178	198	108	0.25	normal	normal
109	51	M		5	134	176	86	0.08	normal	normal

110	49	M		5	143	187	95	0.17	normal	normal
111	57	M		6	156	200	99	0.27	normal	normal
112	45	F		4	243	254	36	0.46	normal	impaired
113	54	M		7	176	201	43	0.33	normal	impaired
114	50	F		4	165	234	89	0.23	normal	normal
115	52	F		7	178	199	100	0.1	normal	normal
116	56	F		6	134	178	96	0.2	normal	normal
117	49	F		9	145	189	91	0.26	normal	normal
118	51	F		8	123	179	88	0.12	normal	normal
119	48	M		9	145	176	33	2.1	normal	impaired
120	59	M		8	176	201	35	3.1	normal	impaired